

truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is
5 matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the
10 query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment
15 of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent
20 identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject
25 sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce
30 silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less

than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., *J. Biotechnology* 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem* 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more

biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show a functional activity (e.g., biological activity) of the polypeptide of the invention of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein or fragments thereof, (e.g., including but not limited to fragments encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having a functional activity of a polypeptide of the invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in the related cDNA clone contained in a

deposited library. the nucleic acid sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, *Science* 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side

chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1

amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the prostate and prostate cancer polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers, for example, to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in a deposited cDNA clone; or is a portion of a polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited cDNA clone; or is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; or is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in a related cDNA clone contained in a deposited library, the nucleotide sequence shown in SEQ ID NO:X or the complementary strand thereto. In this context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-300, 301-350, 351-

- 400, 401-450, 451-500, 501-550, 551-600, 651-700, 701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050,
- 5 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of SEQ ID NO:X, or the complementary strand thereto. In this context
- 10 "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity) of the polypeptide encoded by the polynucleotide of which the sequence is a portion. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which
- 15 hybridize to one or more of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from

20 about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050,

25 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of the cDNA nucleotide sequence contained in the deposited cDNA

30 clone, or the complementary strand thereto. In this context "about" includes the particularly recited range, or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a

functional activity (e.g., biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in the deposited cDNA clone. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA contained in the related cDNA clone contained in a deposited library. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, and 1181 to the end of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained

when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mut⁵ein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in the related cDNA clone contained in a deposited library). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mut³⁰ein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular

polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in deposited cDNA clone referenced in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y), and/or the cDNA in the related cDNA clone contained in a deposited library, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in the related cDNA clone contained in a deposited library may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X, or the cDNA in a deposited cDNA clone may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; <http://www.dnastar.com/>).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions,

Kyte-Doolittle hydrophilic regions and hydrophobic regions. Eisenberg alpha- and beta-amphipathic regions. Karplus-Schulz flexible regions. Emmini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emmini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively consisting of, an amino acid sequence that displays a functional activity of the polypeptide sequence of which the amino acid sequence is a fragment.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of

SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Table 4.

Sequence/ Contig ID	Epitopes
574130	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 941 as residues: Ala-10 to Asp-18, Asp-20 to Cys-27, Cys-44 to Gly-52, Pro-57 to Ser-62, Pro-65 to His-72, Gln-88 to Asn-94, Pro-118 to Thr-127, Pro-129 to Thr-143, Tyr-156 to Tyr-165, Pro-167 to Leu-172, Cys-180 to Asp-185.
637706	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 942 as residues: Arg-1 to Glu-6, Lys-11 to Val-24, Pro-27 to Gln-36, Glu-49 to Gly-54, His-59 to Gly-73, Thr-86 to Ala-97, Pro-104 to Gly-113, Asp-137 to Asp-160, Arg-177 to Asn-195, Leu-203 to Asn-212, Asn-219 to Thr-231, Lys-238 to Tyr-247, Glu-249 to Asn-254, Met-269 to Asp-303, Ser-328 to Ser-336.
684310	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 944 as residues: Ala-13 to Arg-20, Glu-25 to Arg-40.
731016	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 945 as residues: Gly-13 to Leu-20, Glu-40 to Ala-45.
827771	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 946 as residues: Ala-11 to Glu-16.
828193	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 947 as residues: Gly-1 to Glu-9, Ala-15 to Ala-21.
828194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 948 as residues: Pro-45 to Trp-53.
828199	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 949 as residues: Gly-38 to Ser-44, Leu-123 to Trp-138, His-149 to Pro-154.
828221	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 950 as residues: Lys-32 to Leu-41, Arg-119 to Tyr-124, Pro-197 to Arg-204, Asp-236 to Lys-242, Ala-290 to Tyr-296, Thr-320 to Arg-331, Asp-337 to Val-343, His-358 to Gly-368, Thr-419 to Gln-424.
828235	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 951 as residues: Pro-74 to Arg-82.
828236	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 952 as residues: Lys-10 to Gly-15, Pro-22 to Ser-27, Lys-38 to Glu-63, Lys-74 to Val-87, Met-89 to Glu-123, Lys-130 to Glu-196, Val-201 to Ala-207, Arg-251 to Lys-256, Glu-271 to Arg-279, Pro-317 to Asn-327, Lys-382 to Gln-390, Tyr-409 to Glu-415.
828237	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 953 as residues: Ala-6 to Arg-20, Glu-33 to Lys-40, Gln-45 to Leu-50, Arg-52 to Gln-72, Leu-78 to Gln-94, Gln-105 to Gln-114.
828242	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 955 as residues: Thr-1 to Trp-9, Pro-26 to Ala-32, Gly-58 to Arg-68, Gln-73 to Thr-99, Ala-191 to Asp-196, Glu-225 to Glu-234.
828248	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 957 as residues: Lys-21 to Glu-27, Thr-84 to Asp-89, His-103 to Val-109.
828250	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 958 as residues: Glu-106 to Ser-111.
828256	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 959 as residues: Gly-44 to Trp-49, Pro-90 to Ser-95, Tyr-133 to Lys-142, Trp-223 to Glu-242.
828267	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 960 as residues: Pro-1 to His-11, Arg-36 to Gly-52, Arg-62 to Gly-73, Gly-85 to Leu-96, Pro-112 to Gly-117, Ser-130 to Gly-138.
828272	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 962 as residues: Glu-1 to Gly-13, Ser-58 to Phe-65, Thr-118 to Gly-131, Glu-139 to Arg-157.
828273	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 963 as residues: Ser-1 to Pro-6, Gln-38 to Arg-43.
828290	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 964 as residues: Trp-61 to Cys-67.

828326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 965 as residues: Arg-2 to Gln-11, Ala-17 to Ser-24, Arg-45 to Arg-58, Pro-60 to Gly-67, Ser-86 to Thr-92, Asn-143 to Leu-158.
828397	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 966 as residues: Arg-18 to Arg-33.
828405	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 967 as residues: Ser-50 to Leu-57, Ser-88 to Ser-99, Glu-104 to Val-112, Glu-122 to Val-127, Ile-152 to Asp-157.
828461	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 968 as residues: Ala-3 to Ala-16, Leu-25 to Pro-44, Ser-82 to Leu-88, Pro-91 to Arg-99, Pro-110 to Glu-118, Ile-120 to Lys-136, Cys-142 to Leu-149, Glu-156 to Leu-167, Arg-169 to Arg-180, Gly-197 to Pro-212, Arg-269 to Leu-283.
828482	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 969 as residues: Glu-1 to Ser-7.
828491	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 971 as residues: Arg-42 to Asn-48.
828492	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 972 as residues: Pro-28 to Lys-33, Arg-41 to Glu-47.
828494	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 973 as residues: Phe-24 to Val-32, Arg-49 to Val-55, Tyr-59 to Glu-68, Leu-72 to Asn-80.
828496	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 974 as residues: Gly-1 to Arg-8, Ser-17 to Arg-22, Arg-41 to Leu-47, Lys-49 to Lys-57, Leu-66 to Arg-73, Glu-94 to Thr-104, Arg-117 to Leu-126, Lys-184 to Asn-193, Glu-197 to Arg-216.
828498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 975 as residues: Glu-62 to Leu-68, Ile-104 to Ser-111.
828504	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 976 as residues: Ser-14 to Pro-21.
828512	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 978 as residues: Asn-26 to Gln-36, Val-48 to Asp-62, Lys-112 to Ser-123, Val-127 to Phe-132, Phe-139 to Asp-151, Val-158 to Glu-180.
828516	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 979 as residues: Gly-14 to Gly-20, Ala-22 to Ala-33, Arg-83 to Thr-88, Arg-109 to Leu-105, Lys-130 to Lys-141.
828519	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 980 as residues: Gly-7 to Pro-13, His-20 to Ala-25.
828521	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 981 as residues: Asn-13 to His-19, Ser-37 to Arg-45.
828522	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 982 as residues: Lys-12 to Glu-19, Glu-38 to Gly-43, Pro-82 to Lys-93.
828525	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 983 as residues: Pro-23 to Pro-30, Ala-59 to Ser-64, Pro-84 to Thr-93, Pro-135 to Gly-140.
828529	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 984 as residues: Ser-15 to Gln-20, Gln-92 to Phe-113, Thr-141 to Gly-146, Val-153 to Thr-158.
828530	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 985 as residues: Pro-5 to Gln-15, Lys-23 to Leu-32.
828536	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 986 as residues: His-28 to Glu-34.
828537	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 987 as residues: Ile-28 to Leu-33, Gln-42 to Ser-52, Ser-54 to Trp-59.
828539	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 988 as residues: Ala-1 to Leu-9, Ser-19 to Thr-31.
828540	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 989 as residues: Arg-1 to Lys-12, Gly-17 to Ile-23.
828543	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 991 as residues: Ala-13 to Gln-20, Asp-33 to Asn-39.
828544	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 992 as residues: Val-15 to Asp-21.
828551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 995 as residues:

	Met-12 to Pro-17.
828560	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 998 as residues: Val-8 to Arg-17.
828561	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 999 as residues: Asn-7 to Gly-20, Thr-32 to Tyr-37, Arg-57 to Gly-66.
828565	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1000 as residues: Arg-1 to Asn-18.
828566	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1001 as residues: Arg-41 to His-50, Lys-52 to Thr-60.
828567	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1002 as residues: Gln-7 to Cys-12, Pro-20 to Lys-30.
828568	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1003 as residues: Pro-10 to Glu-20, Asn-29 to Trp-37, Ala-44 to Arg-51, Gln-69 to Gly-79.
828570	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1005 as residues: Ser-16 to Leu-24.
828571	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1006 as residues: Leu-1 to Gln-17.
828574	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1007 as residues: Pro-117 to Lys-134, Gln-136 to Trp-143.
828575	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1008 as residues: Lys-6 to Ala-13.
828578	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1010 as residues: Gly-72 to Asp-81, Cys-89 to Gly-100, Lys-107 to Arg-114, Lys-119 to Gln-126, Arg-140 to Ser-160.
828580	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1011 as residues: Pro-1 to Ala-7, Lys-54 to Gln-68, Leu-81 to Gln-93.
828581	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1012 as residues: Glu-13 to Ser-21, Glu-31 to Glu-37, Lys-53 to Ala-60.
828583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1013 as residues: Gln-1 to Gly-7, Thr-22 to Gly-31.
828585	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1014 as residues: Leu-28 to His-34.
828587	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1015 as residues: Gln-1 to Lys-8, Ser-25 to Phe-38, Thr-79 to Val-90, Arg-118 to Glu-125.
828592	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1017 as residues: Gln-12 to Gln-17, Arg-43 to Gln-49, Lys-62 to Lys-67, Glu-78 to Gly-83.
828594	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1019 as residues: Glu-9 to Gln-18.
828596	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1020 as residues: Thr-1 to His-8.
828597	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1021 as residues: Gln-12 to Trp-17, Asp-83 to Ile-97, Gln-99 to Asp-104, Thr-210 to Ser-216, Arg-279 to Thr-296.
828598	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1022 as residues: Thr-1 to Ser-7.
828601	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1023 as residues: Ile-1 to Trp-10, Thr-32 to Ser-38, Pro-49 to Gly-56, Ser-78 to Arg-83, Phe-113 to Arg-122, Leu-156 to Asp-173.
828605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1024 as residues: Arg-6 to Pro-12.
828608	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1025 as residues: Arg-52 to Ile-59, Asp-65 to Phe-76, Lys-96 to Leu-102.
828609	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1026 as residues: Gly-29 to Gly-36, Lys-105 to Thr-112, Phe-134 to Asn-145, Pro-182 to Gly-190.
828610	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1027 as residues: Pro-49 to Asp-58, Lys-60 to Ile-66, Ser-68 to Glu-76, Val-95 to Asn-101, Lys-118 to Thr-124.
828617	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1028 as residues:

	Ser-14 to Arg-22, Leu-24 to Cys-30, Pro-35 to Gly-40.
828620	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1029 as residues: Leu-2 to Arg-10, Ala-57 to Lys-64, Lys-81 to Leu-88, Tyr-160 to Pro-169, Met-203 to Asp-216.
828623	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1032 as residues: His-38 to His-44.
828625	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1033 as residues: Ile-19 to Asn-28.
828635	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1035 as residues: Arg-3 to Arg-10.
828637	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1036 as residues: Asp-9 to Cys-15.
828639	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1037 as residues: Pro-13 to His-20.
828645	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1038 as residues: Glu-1 to Gly-10, Lys-18 to Arg-41, Ala-55 to Pro-65.
828648	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1039 as residues: Ala-12 to Asn-20, Pro-23 to Asn-28, Phe-47 to Val-52, Lys-88 to Gly-93, Tyr-113 to Asn-123.
828649	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1040 as residues: Pro-14 to Gln-29.
828651	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1041 as residues: Gly-2 to Lys-13.
828655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1043 as residues: Val-13 to Trp-27.
828657	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1044 as residues: Glu-20 to Leu-30, Glu-79 to Gly-84, Asp-89 to Trp-96.
828660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1045 as residues: Pro-37 to Thr-43.
828663	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1046 as residues: Ala-19 to Gly-24.
828666	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1047 as residues: His-54 to Gly-59.
828668	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1048 as residues: Pro-1 to Gly-12, Pro-30 to Leu-48.
828669	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1049 as residues: Pro-2 to Ser-7, Trp-27 to Lys-38.
828671	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1051 as residues: Asp-89 to Ile-94.
828672	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1052 as residues: Lys-16 to Ser-23.
828675	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1053 as residues: Lys-11 to His-16, Ala-26 to Ser-65.
828677	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1054 as residues: Pro-7 to Trp-13.
828678	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1055 as residues: Glu-188 to Arg-196.
828679	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1056 as residues: Asn-17 to Lys-23.
828680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1057 as residues: Pro-7 to Glu-17, Ser-68 to Tyr-85, Ser-94 to Asn-101, Thr-122 to Arg-129, Ser-169 to Val-174.
828681	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1058 as residues: Asp-1 to Asp-19, Arg-27 to Leu-33.
828682	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1059 as residues: Pro-34 to Glu-39, Ala-41 to Gly-47, Glu-100 to Ser-111.
828683	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1060 as residues: Gly-7 to Val-14.
828686	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1061 as residues:

	Pro-15 to Glu-20, Gln-71 to Leu-84, Glu-86 to Ser-96, Glu-116 to Pro-121, Val-176 to Leu-196, Asn-216 to Ala-224.
828687	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1062 as residues: Glu-3 to Ala-13, Ile-22 to Ser-28.
828688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1063 as residues: Asp-7 to Ala-15, Pro-34 to Ile-60, Gln-110 to Asn-117.
828689	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1064 as residues: Ser-74 to Met-96, Leu-108 to Trp-117, Gly-126 to Gly-131, Glu-161 to Asp-178, Lys-181 to Tyr-191, Arg-196 to Ser-202.
828692	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1065 as residues: Pro-73 to Thr-86, Ser-93 to Val-102, Ala-157 to Lys-162, Thr-169 to Lys-184, Asp-198 to Tyr-211.
828694	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1067 as residues: Thr-1 to Ala-10, Pro-18 to Arg-25, Ala-49 to Leu-56, Ser-104 to Arg-111.
828696	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1068 as residues: Ser-5 to Ser-10.
828699	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1070 as residues: Asp-7 to Val-17, Ala-21 to Ser-26.
828702	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1071 as residues: Val-14 to Gly-26, Ser-76 to His-87, Ile-127 to Phe-134, Pro-151 to Asn-157.
828703	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1072 as residues: Cys-58 to Ser-66.
828704	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1073 as residues: Thr-35 to Thr-42.
828706	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1074 as residues: Arg-1 to Glu-13.
828708	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1075 as residues: Asn-17 to Pro-27, Ser-46 to His-51, Leu-53 to Asp-60, Cys-62 to Ile-68.
828711	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1076 as residues: Asp-24 to Phe-31.
828712	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1077 as residues: Ser-44 to Lys-49, Glu-65 to Lys-76.
828713	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1078 as residues: Pro-1 to Asp-6, Arg-13 to Gly-26.
828714	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1079 as residues: Pro-24 to Glu-42, Gln-58 to Asp-64, Gln-80 to His-90, Pro-92 to Asp-103, Tyr-139 to Glu-153, Asp-162 to Asp-180, Glu-189 to Phe-200, Ser-203 to Gln-213, Glu-219 to Gly-224, Lys-227 to Ser-236, Pro-241 to Asn-260, Phe-275 to Ser-281, Phe-305 to Asn-314, Gln-319 to Tyr-329, Thr-341 to Ser-357, Pro-360 to Cys-365, Trp-384 to Phe-398, Gln-401 to Lys-410.
828718	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1081 as residues: Asp-70 to Leu-85, Ser-195 to Arg-205, Arg-262 to Ala-268, Asn-270 to Ala-277.
828728	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1084 as residues: Gly-12 to Val-19, Asp-38 to Gln-55, Gln-84 to Tyr-91, Gln-96 to Asp-102.
828730	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1085 as residues: Gly-142 to Arg-148, Ser-173 to Gln-178, Thr-202 to Ile-207, Leu-276 to Val-282, Pro-321 to Gly-353, Thr-355 to Glu-364, Glu-380 to Lys-385.
828732	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1086 as residues: Leu-8 to Lys-29, Leu-79 to Glu-86, Asn-106 to Trp-113.
828733	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1087 as residues: Lys-26 to Lys-33.
828735	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1088 as residues: Ser-10 to Pro-21, Ser-94 to Ala-111, Ala-125 to Met-142, Pro-144 to Gln-150, Asp-194 to Asn-201, Val-216 to Arg-243.
828740	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1091 as residues: Asn-12 to Leu-21, Leu-23 to Ser-28.
828742	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1092 as residues:

	Ser-149 to Leu-158
828748	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1093 as residues: Pro-21 to Lys-31, Glu-46 to Thr-52, Cys-93 to Trp-100, Glu-144 to Gln-150, Gln-171 to Ser-180, Pro-205 to Trp-210, Ser-222 to Cys-228.
828752	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1095 as residues: Pro-23 to Gly-28, Ser-34 to Gly-39, Leu-44 to Arg-56, Gln-101 to Leu-112, Leu-119 to Ser-124, Lys-129 to Trp-138.
828753	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1096 as residues: Ile-1 to Gly-44.
828754	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1097 as residues: Leu-21 to Gln-27.
828757	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1098 as residues: Thr-27 to Arg-34, Tyr-40 to Trp-47, Thr-83 to Ser-90.
828761	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1099 as residues: Arg-1 to Gln-19.
828762	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1100 as residues: Phe-1 to Arg-11, Leu-48 to Lys-56.
828764	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1101 as residues: Asp-79 to Arg-84.
828765	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1102 as residues: Ala-5 to Ala-10.
828766	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1103 as residues: Gly-1 to Lys-10, Glu-21 to Leu-27, Ser-38 to Leu-43.
828768	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1105 as residues: Lys-39 to Lys-64.
828770	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1106 as residues: Ser-3 to Tyr-9.
828771	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1107 as residues: Ser-13 to Cys-21.
828772	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1108 as residues: Arg-28 to Asp-34.
828776	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1111 as residues: Pro-6 to Thr-13.
828784	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1118 as residues: Glu-6 to Leu-21, Ala-34 to Ala-40.
828785	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1119 as residues: Arg-53 to Ser-64.
828786	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1120 as residues: Thr-1 to Thr-16, Ser-32 to Lys-39.
828790	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1122 as residues: Pro-13 to Ala-21.
828791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1123 as residues: Lys-1 to Cys-6.
828792	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1124 as residues: Arg-1 to Thr-7, Gln-12 to Gly-17.
828799	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1128 as residues: Thr-2 to Lys-8, Val-47 to Trp-52.
828802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1130 as residues: Gly-41 to Met-47, Lys-59 to Arg-72.
828803	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1131 as residues: Arg-8 to Thr-14, Ala-51 to Ser-58, Ser-60 to Ser-79, Leu-97 to His-104.
828804	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1132 as residues: Lys-1 to Pro-12, Asn-43 to Lys-48.
828805	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1133 as residues: Glu-15 to Ser-20, Thr-28 to Arg-39.
828807	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1134 as residues:

	Glu-14 to Lys-19.
828821	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1142 as residues: Cys-9 to Leu-15, His-28 to Gly-36.
828825	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1145 as residues: Pro-38 to Pro-43.
828826	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1146 as residues: Ile-7 to Leu-15, Lys-18 to Ser-36, Thr-66 to Lys-72, Thr-91 to Tyr-97, Val-99 to Cys-106, Glu-154 to Lys-159, Glu-171 to Asn-176, Met-187 to Ser-192, Leu-203 to Gln-212.
828829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1147 as residues: Ser-52 to Glu-58.
828835	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1150 as residues: Lys-89 to Ser-104.
828838	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1151 as residues: Arg-1 to Arg-11.
828840	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1152 as residues: Gly-32 to Gly-37.
828845	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1153 as residues: Asn-23 to Tyr-34.
828846	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1154 as residues: Ala-40 to Tyr-55, Glu-57 to Asn-66, Glu-74 to Asn-79.
828847	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1155 as residues: Gln-66 to Gly-77, Gly-86 to Ala-93.
828849	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1156 as residues: Arg-16 to Ser-25, Asp-97 to Pro-106, Pro-166 to Leu-176, Glu-271 to Gln-285, Thr-287 to Met-294, Ser-310 to Glu-316, Pro-330 to Gly-338, Phe-400 to Ser-415, Thr-425 to Ser-433, Lys-453 to Pro-469.
828852	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1158 as residues: Val-33 to Ser-39.
828853	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1159 as residues: Pro-25 to Ser-31, Ser-34 to Gly-41.
828857	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1160 as residues: Lys-5 to Leu-10, Ser-20 to Glu-30, Leu-32 to Thr-37.
828861	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1161 as residues: Arg-33 to Phe-38, Arg-59 to Gly-64, Pro-100 to His-121, Arg-144 to Pro-162, Gln-213 to Thr-221, Pro-262 to Trp-268, Ala-292 to Phe-302, Pro-315 to Pro-323.
828866	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1162 as residues: Cys-1 to Gln-6, Gln-79 to Ala-89, Thr-96 to Leu-102.
828872	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1163 as residues: Gly-17 to Leu-40, Ala-47 to Phe-63, Glu-66 to Val-71, Ile-75 to His-92, Glu-112 to Asn-119, Asp-122 to Arg-135, Asn-140 to Phe-152, Asn-160 to Arg-166.
828874	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1164 as residues: Arg-1 to Ala-34, Pro-41 to Pro-47, Pro-49 to Asp-57, Asn-99 to Ala-105, Met-107 to Thr-112, Lys-118 to Ser-135, Glu-145 to Ile-156, Ala-202 to Lys-209, Lys-214 to Ile-220, Ala-224 to Ala-236, Ala-239 to Pro-248, Pro-260 to Lys-270, Lys-275 to Lys-300.
828875	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1165 as residues: Pro-17 to Gly-24, His-31 to Phe-36, Gly-72 to Val-79, Val-99 to Asp-104.
828878	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1167 as residues: Ser-33 to Asp-45, Thr-48 to Glu-53, Lys-70 to Glu-75, Phe-125 to Phe-131, Asp-216 to Ile-223, Met-244 to Thr-252, Asn-272 to Leu-281, Gln-314 to Lys-320, Ala-340 to Ser-348.
828879	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1168 as residues: Ser-1 to Arg-8.
828881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1169 as residues: Arg-1 to Lys-8, Asp-184 to Gly-190, Pro-269 to Asp-274.
828885	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1170 as residues: Glu-6 to Gly-11, Gln-34 to Ala-41, Val-62 to Gly-69, Val-79 to Glu-92, Pro-95 to Asp-100, Lys-106 to Leu-123, Asp-178 to Asn-185, His-208 to Ser-213, Glu-224 to Val-231, Gly-233 to Lys-

	241. Ser-254 to Ser-265, Phe-279 to Ser-285, Asn-292 to Gly-307, Lys-311 to Gly-324.
828887	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1172 as residues: Ala-1 to Lys-6, Ala-55 to Ser-60, Tyr-65 to Tyr-70, Thr-75 to Pro-84, Ser-106 to Ser-111, Asn-121 to Arg-131, Glu-145 to Pro-150, Pro-156 to His-171, Ser-188 to Leu-196, Asp-231 to His-238, Ser-276 to Arg-281, Arg-298 to Glu-307, Glu-332 to Glu-339, Tyr-355 to Thr-362, Ala-381 to Ser-392, Glu-409 to Val-422.
828891	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1174 as residues: Pro-1 to Glu-18, Gly-26 to Pro-33, Pro-66 to Gly-75, Gln-105 to Val-110, Ser-128 to Pro-134, Glu-182 to Leu-187.
828899	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1175 as residues: His-1 to Arg-11, Ser-40 to Gln-49.
828907	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1176 as residues: Ser-21 to Asp-28, Pro-30 to Cys-38, Arg-98 to His-103, Asn-118 to Ile-136, Ser-153 to Trp-161, Arg-163 to Tyr-172, Thr-174 to Ser-181.
828917	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1179 as residues: His-1 to Gln-22, Thr-27 to Phe-38.
828921	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1180 as residues: Glu-1 to Glu-6.
828922	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1181 as residues: Thr-6 to Ser-21.
828926	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1184 as residues: Gly-108 to Tyr-117.
828928	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1185 as residues: Gln-7 to Trp-13, Pro-46 to Ala-55.
828930	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1186 as residues: Glu-73 to His-79, Gly-105 to Tyr-110, Asp-161 to Asn-166, Lys-187 to Gln-196, Tyr-200 to Leu-206, Glu-222 to Met-229, Ala-252 to Ser-267, Asn-314 to Trp-323, Gly-344 to Asn-352.
828937	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1188 as residues: Met-28 to Lys-33, Asp-40 to Ala-64, Tyr-72 to Lys-85, Thr-124 to Leu-131, Ala-148 to Tyr-155.
828940	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1189 as residues: Pro-23 to Gln-29, Ile-56 to Asn-61, Lys-69 to Lys-75.
828943	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1191 as residues: Val-5 to Gly-11, Gln-26 to Asp-36, Val-93 to Lys-98, Lys-101 to Thr-124, Lys-130 to Asp-141, Thr-163 to Lys-172, Ser-195 to Ala-200, Tyr-210 to Ile-220.
828946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1192 as residues: Arg-29 to Glu-34, Ala-74 to Leu-79, Ser-88 to Ala-96, Glu-126 to Leu-133, Glu-149 to Pro-156, Pro-177 to Asp-182.
828947	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1193 as residues: Lys-28 to Gly-40.
828956	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1194 as residues: Pro-84 to Asp-94, Ile-99 to Asn-105, Lys-131 to Lys-136, Lys-141 to Asn-146, Lys-153 to His-162, Asp-170 to Arg-179, Gln-248 to Ile-258, Thr-262 to Leu-267, Thr-270 to Phe-279, Arg-294 to Leu-302.
828958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1195 as residues: Cys-14 to Ser-25.
828965	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1196 as residues: Ala-29 to Leu-35, Pro-83 to Val-88.
828969	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1197 as residues: Arg-2 to Gly-8.
828971	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1198 as residues: Glu-53 to Lys-60.
828973	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1199 as residues: Ser-18 to Thr-25, His-177 to Tyr-186.
828980	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1200 as residues: Cys-4 to Glu-15.
828984	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1201 as residues:

	Asn-14 to Lys-19, Asp-55 to Lys-64, Thr-120 to Glu-125, Pro-149 to Gly-154, His-206 to Lys-213, Pro-242 to Arg-249, Met-269 to Glu-279, Arg-281 to Ser-287, Phe-312 to Gly-317, Arg-361 to Ser-368, Glu-374 to Gln-380, Ile-386 to Tyr-391, Glu-412 to Gln-428, Arg-435 to Val-471, Ser-483 to Lys-502, Lys-507 to Glu-517, Lys-519 to Pro-530, Ser-541 to Pro-550, Gly-567 to Lys-589, Glu-593 to Val-613, Lys-616 to Leu-636, Ser-647 to Leu-673, Pro-677 to Glu-689.
828988	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1203 as residues: Asp-60 to Lys-75.
828995	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1205 as residues: Thr-26 to Gly-33, Ser-42 to Ser-53, Pro-73 to Leu-78, Pro-101 to Gly-107, Pro-147 to Ser-157, Pro-168 to Ser-176, Ser-203 to His-208, Ser-216 to Cys-221.
829005	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1207 as residues: Pro-17 to Glu-22, Thr-129 to Lys-137, Asp-164 to Asp-170.
829009	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1208 as residues: Pro-1 to Arg-14, Pro-36 to Arg-54, Arg-61 to His-68, Arg-83 to Ile-92, Ala-95 to Arg-103, Arg-107 to Glu-114.
829012	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1210 as residues: His-6 to Ser-11, Ser-122 to Asn-128, Leu-216 to Asp-221, Ser-323 to His-328.
829013	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1211 as residues: Ile-10 to Leu-16, Pro-24 to Cys-29.
829019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1212 as residues: Tyr-29 to Ser-42.
829020	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1213 as residues: Pro-22 to Arg-32, Leu-122 to Asp-127, Gln-134 to Tyr-140, Asp-153 to Arg-168.
829021	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1214 as residues: Ile-11 to Phe-16, Pro-38 to Ile-53.
829030	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1216 as residues: Lys-82 to Gly-87, Lys-224 to Asp-230, His-245 to Glu-253, Ser-279 to Lys-285, Val-308 to Lys-314, Arg-342 to Met-348, Lys-392 to Arg-397, His-452 to Gly-458.
829035	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1217 as residues: His-36 to Ser-43.
829051	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1221 as residues: Pro-3 to Trp-9.
829052	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1222 as residues: Ala-32 to Pro-37, Pro-57 to Trp-62, Pro-82 to Leu-93.
829057	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1223 as residues: Glu-9 to Thr-21, Leu-32 to Arg-45, Glu-49 to Ala-54, Lys-62 to Leu-68, Ala-71 to Thr-99, Leu-106 to Glu-113.
829059	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1225 as residues: Asn-2 to Ser-16.
829061	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1226 as residues: Lys-1 to Ser-7.
829062	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1227 as residues: Pro-15 to Cys-23, Pro-46 to Ala-54, Pro-71 to Gly-78, Leu-84 to Pro-92, Leu-131 to Arg-137, Ala-151 to Glu-161, Thr-215 to Leu-222, Glu-253 to Ser-261, Leu-269 to Leu-275, Asn-280 to Ser-285, Arg-292 to Glu-298, Gly-302 to Ser-309, Thr-322 to Arg-327, Lys-376 to Leu-388.
829063	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1228 as residues: Gly-12 to Ala-20, Arg-58 to Phe-68.
829064	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1229 as residues: Cys-9 to Tyr-14, Gly-35 to Thr-41, Ser-44 to Thr-49, Cys-53 to Thr-68, Leu-98 to Val-103, Ile-180 to Tyr-187, Ser-208 to Val-215.
829066	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1230 as residues: Phe-15 to Met-20.
829069	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1232 as residues: Asn-1 to Gly-12, Pro-31 to His-38, Ser-54 to Ser-59, Gly-64 to Lys-69.
829074	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1233 as residues: Leu-1 to Thr-17, Glu-38 to Gln-44, Glu-46 to Asp-55, Glu-82 to Glu-100, Lys-119 to Gly-129.

	Lys-147 to Ser-153, Pro-187 to Thr-210, Leu-225 to Val-233, Pro-272 to Gly-279, Arg-290 to Ser-303, Pro-311 to Lys-318, Ser-334 to Pro-356, Ser-370 to Arg-377, Gly-407 to Ser-412, Met-415 to His-423.
829077	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1234 as residues: Thr-1 to Thr-10, Asp-29 to Trp-35, His-37 to Trp-50, Lys-58 to Thr-65, Glu-77 to Glu-91, Glu-116 to Arg-128, Cys-219 to Pro-224.
829085	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1237 as residues: Arg-9 to Lys-31, Leu-66 to Lys-71, Gln-119 to Gly-131, Gln-230 to Leu-239.
829093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1238 as residues: Gln-21 to Asp-26, Glu-178 to Asn-185, Arg-213 to Glu-218, Asp-238 to Asn-246, Val-264 to Pro-272, Val-280 to His-288.
829099	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1239 as residues: Arg-2 to Ser-8, Thr-140 to Ser-151, Val-153 to His-165, Leu-176 to Arg-182, Asp-200 to Thr-207, Asn-224 to Asp-229, Cys-239 to Ser-246.
829102	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1241 as residues: Pro-10 to Lys-19.
829103	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1242 as residues: Pro-30 to His-46, Glu-127 to Leu-133.
829104	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1243 as residues: Ser-19 to Trp-26, Lys-37 to Leu-59.
829109	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1244 as residues: Gln-22 to Ser-29.
829115	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1246 as residues: Gly-23 to Cys-29, Pro-35 to Cys-40, Gly-51 to Ser-64, Asp-108 to Arg-115, Glu-132 to Val-146, Thr-149 to Glu-155.
829120	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1249 as residues: Gly-68 to Arg-74, Pro-83 to Asn-88.
829126	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1252 as residues: Lys-18 to Lys-28.
829136	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1254 as residues: Asp-19 to His-26, Asp-127 to Gly-144, Thr-179 to Gln-194, Val-223 to Thr-229, Pro-235 to Tyr-240.
829138	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1255 as residues: Ala-23 to Glu-28, Glu-37 to Ser-46, Glu-63 to Gly-68, Gln-75 to Phe-84, Thr-91 to Ser-97, His-106 to Pro-117.
829142	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1256 as residues: Pro-21 to Thr-35.
829148	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1257 as residues: Pro-33 to Lys-40.
829149	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1258 as residues: His-9 to Glu-18, Arg-91 to Gly-96, Ser-124 to Asp-133, Asn-163 to Cys-172, Asn-216 to Thr-222, Thr-229 to Ile-235, Lys-238 to Glu-243.
829162	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1260 as residues: Arg-1 to Arg-6, Ala-53 to Gln-58.
829179	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1263 as residues: Gln-10 to Thr-21.
829184	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1264 as residues: Thr-76 to Val-81, Leu-88 to Pro-100, Tyr-140 to Lys-150.
829185	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1265 as residues: Pro-1 to Ser-21.
829188	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1266 as residues: Lys-11 to Trp-20, Ser-22 to Ala-27, Ile-35 to Met-51, Val-53 to Glu-69, Asn-145 to Leu-151, Asp-179 to Gln-187, Pro-280 to Ala-285, Asp-293 to Ile-300.
829190	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1267 as residues: Arg-3 to Gln-9, Pro-29 to Gln-34, Glu-98 to Asp-111.
829196	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1269 as residues:

	Leu-53 to Asn-62, Ala-125 to Ala-132.
829197	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1270 as residues: Leu-14 to Pro-19, Ser-25 to Ser-37.
829203	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1272 as residues: Gly-1 to Leu-9, Ser-80 to Gly-85.
829209	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1273 as residues: Ser-17 to Glu-29.
829210	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1274 as residues: Ser-13 to Tyr-18.
829214	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1275 as residues: Pro-2 to Asn-10, Lys-49 to Asn-54, Arg-91 to Asn-96, Glu-118 to Cys-125, Pro-139 to Gly-144.
829215	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1276 as residues: Asn-1 to Leu-6, Ser-27 to Pro-32.
829219	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1277 as residues: Pro-15 to Pro-25, Ala-54 to Phe-61, Ile-63 to Ser-82.
829220	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1278 as residues: Pro-1 to Ser-9, Glu-48 to Gly-54, Gly-66 to Leu-71, Pro-78 to Glu-84, Ala-108 to Gln-116, Ile-167 to Asp-172, Thr-179 to His-185.
829222	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1279 as residues: Thr-45 to Gln-51, Cys-53 to Asp-60, Gly-122 to Gly-127, Lys-136 to Gly-142, Pro-164 to Lys-172.
829223	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1280 as residues: Ile-11 to Trp-16.
829225	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1281 as residues: Lys-24 to Trp-30.
829226	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1282 as residues: Lys-48 to Lys-56, Arg-64 to Glu-79, Glu-102 to Tyr-111, Glu-159 to Cys-165, Thr-187 to Lys-193, Tyr-212 to Arg-220, Tyr-254 to Pro-262, Gly-278 to Asp-284, Pro-336 to Pro-341, Pro-441 to Gly-452, Glu-468 to Asp-480, Phe-486 to Tyr-495, Asp-498 to Asn-503.
829227	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1283 as residues: Pro-40 to Ala-46.
829231	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1284 as residues: Cys-12 to Ser-17.
829233	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1286 as residues: Pro-5 to Met-16, Ala-37 to Ala-46, Pro-70 to Leu-75.
829239	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1287 as residues: Glu-63 to Arg-70, Pro-82 to Leu-91, Arg-139 to Gln-146.
829242	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1289 as residues: Arg-11 to Gly-17, Lys-113 to Gly-120, Arg-163 to Ser-168, Asp-200 to His-210, Ile-217 to Ile-223, Arg-260 to Glu-266, Ser-274 to Leu-281.
829246	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1290 as residues: Arg-17 to Phe-25, Asn-27 to Asn-41, Thr-57 to Ser-69, Gln-92 to Asp-98.
829250	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1291 as residues: Ser-2 to Ile-16.
829253	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1292 as residues: Arg-10 to Arg-20, Gly-48 to Val-53, Glu-69 to Asp-76, Glu-116 to Glu-122, Glu-132 to Trp-143, Asp-166 to Asn-175, Arg-191 to Asn-197, Gln-205 to Gly-233, Lys-235 to Ala-274.
829263	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1294 as residues: Pro-1 to Arg-13, Gly-20 to Gly-27, Gly-32 to Lys-38.
829266	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1295 as residues: Lys-1 to Arg-6.
829271	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1296 as residues: Ala-7 to Thr-13, Lys-56 to Lys-66, Pro-81 to Asp-88, Glu-140 to Thr-148, Ser-158 to Gln-164, Gly-201 to Asp-207, Glu-221 to Ser-230, Pro-236 to Gly-241, Pro-243 to Arg-261, Gln-270 to Gly-286.
829273	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1297 as residues:

	Ser-19 to Ala-24.
829274	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1298 as residues: Pro-58 to Ser-64.
829276	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1299 as residues: Arg-5 to Glu-38.
829280	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1301 as residues: Ser-31 to Arg-36, Gln-61 to Lys-66.
829284	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1303 as residues: Arg-1 to Thr-7, Ala-9 to Arg-14, Gly-24 to Gly-29, Gly-52 to Ala-60, Arg-62 to Gly-71, Arg-84 to Asn-96, Pro-102 to Thr-107.
829287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1305 as residues: Gln-38 to Lys-45.
829295	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1306 as residues: Pro-1 to Lys-13, Ala-32 to Gln-44.
829296	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1307 as residues: Glu-45 to Glu-59, Phe-61 to His-67, Ala-78 to Ser-85, Trp-100 to Pro-105.
829298	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1309 as residues: Phe-4 to Gln-10.
829302	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1310 as residues: Ser-17 to Trp-22, Ser-73 to Arg-80.
829320	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1312 as residues: Val-5 to Lys-18, Val-56 to Lys-64, Pro-94 to Gly-100, Phe-140 to Met-148, Glu-154 to Asp-161, Pro-182 to Cys-188, Pro-190 to Asn-197, Ala-216 to Leu-224.
829322	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1313 as residues: Pro-14 to Lys-26, Asp-31 to Lys-39, Arg-112 to Ile-120, Arg-128 to Gly-141, Lys-144 to Asp-151, Lys-159 to Gly-165, His-187 to Trp-203, Asn-246 to Ala-251, Ala-261 to Gln-266, Glu-271 to Thr-280.
829355	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1314 as residues: Ala-26 to Leu-33, Arg-120 to Phe-126, Thr-191 to Asn-203, Ser-223 to Pro-232.
829364	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1315 as residues: Arg-9 to Leu-15, Leu-67 to Ser-74, Asp-93 to Tyr-98, Leu-101 to Pro-108, Lys-117 to Thr-123, Thr-138 to Leu-143.
829946	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1319 as residues: Pro-20 to Gly-29, Gly-46 to Thr-56.
829952	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1321 as residues: Pro-11 to Glu-34, Leu-82 to Gln-88.
829954	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1322 as residues: Leu-32 to Val-38, Gly-75 to Ser-83, Ser-86 to Tyr-92, Lys-96 to His-104, Ser-109 to Ser-117, Gln-124 to Ser-130, Asn-132 to Asn-141, Pro-164 to Leu-178, His-187 to Gly-194, Pro-203 to Gln-217.
829955	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1323 as residues: Asp-39 to Gly-45, Asn-53 to Arg-80, Gln-85 to Gly-95, Glu-101 to Glu-111, His-132 to Gly-151, Leu-159 to Tyr-166, Ser-174 to Ser-179, His-188 to Gly-200, Gln-226 to Gly-235, Cys-255 to Gly-263.
829957	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1324 as residues: Gly-1 to Phe-12, Thr-14 to Val-22, Arg-30 to Met-37, Arg-63 to Pro-69, Arg-82 to Tyr-95, Glu-102 to Gly-109, Lys-223 to Leu-240.
829958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1325 as residues: Arg-13 to Trp-31, Val-61 to Asn-67, Lys-87 to Arg-92, Leu-97 to Asp-109, Ser-129 to Asp-139.
829960	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1326 as residues: Ile-1 to Ser-10, Ile-26 to Pro-31, Lys-83 to Asp-89, Gly-96 to Asn-101, Pro-122 to Asn-127, Ser-224 to Ile-231, Asp-350 to Pro-356.
829966	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1327 as residues: Tyr-7 to Tyr-15, Pro-43 to Ala-52, Gln-57 to Ala-62, Asn-68 to Ala-73, Tyr-75 to Met-83.
829981	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1330 as residues: Ala-96 to Lys-111, Cys-117 to Cys-128.

829985	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1331 as residues: Arg-11 to Val-19, Ala-21 to Trp-26, Tyr-54 to Lys-76, His-107 to Gln-112.
829988	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1333 as residues: Leu-32 to Glu-43, Gly-50 to Arg-58.
829990	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1334 as residues: Ser-27 to Ser-34, Gly-41 to Val-46.
829991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1335 as residues: Leu-15 to Gln-25.
829992	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1336 as residues: Asp-1 to Gly-8, Lys-26 to Trp-33, Pro-49 to Pro-54.
829993	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1337 as residues: Leu-3 to Ser-9.
829998	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1338 as residues: Glu-42 to Leu-47, Glu-125 to Ala-136.
830001	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1341 as residues: Gly-1 to Met-8, Ile-12 to Pro-17, Gly-77 to Ser-92.
830010	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1344 as residues: Cys-1 to Ser-6, Ala-55 to Ala-65, Pro-92 to Asn-97, Gln-100 to Pro-106, Gly-119 to Gly-125, Leu-135 to Arg-143, Ser-151 to Asp-159, Gln-164 to Ser-169, Thr-180 to Asn-186, Ser-204 to Val-216, Pro-224 to Arg-250, His-275 to Tyr-287.
830128	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1346 as residues: His-4 to Thr-10.
830129	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1347 as residues: Trp-52 to Thr-58, Arg-222 to Gly-227, Asn-255 to Asp-265, Pro-452 to Arg-458, Glu-503 to Lys-509, Gly-556 to Asn-563, Asp-628 to Glu-633, Glu-676 to Ser-697, Ala-708 to Ser-714.
830140	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1349 as residues: Gln-61 to Lys-67.
830157	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1350 as residues: Pro-1 to Arg-7, Arg-14 to Glu-24.
830195	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1351 as residues: Ser-2 to Arg-14, Ala-37 to Lys-45, Glu-60 to Leu-68, His-75 to Glu-82, Arg-92 to Ser-99, Gly-105 to Gln-110, Arg-119 to Phe-125.
830196	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1352 as residues: Lys-15 to Val-27, Glu-47 to Ile-79, Gly-83 to Phe-133, Lys-135 to Glu-142, Glu-174 to Ile-182, Ala-249 to Lys-257, Glu-272 to Leu-280, His-287 to Glu-294, Arg-304 to Ser-311, Gly-317 to Gln-322, Leu-372 to Lys-388, His-404 to Leu-409.
830409	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1353 as residues: Ser-4 to Ala-9.
830417	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1354 as residues: Pro-33 to Leu-39, Glu-54 to Val-59, Gly-69 to Ser-76.
830531	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1355 as residues: Lys-29 to Glu-37, Leu-126 to Gly-131, Asp-149 to Glu-159, Pro-235 to Thr-255.
830677	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1356 as residues: Leu-23 to Val-37, Glu-39 to Asp-51, Gly-66 to Arg-71, Gly-79 to Gly-85, Pro-87 to Leu-94, Gly-102 to Lys-123, Ser-135 to Asp-142, Gln-145 to Arg-158, Gln-169 to Glu-174, Ala-178 to Gln-190, Ala-196 to Glu-209, Glu-212 to Glu-220, Arg-249 to His-255, Ala-298 to Glu-309, Arg-314 to Lys-368.
831355	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1357 as residues: Lys-49 to Gln-55, Glu-83 to Lys-90, Gly-158 to Gly-164, Lys-185 to Gly-192.
831420	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1358 as residues: Ala-6 to His-19, Glu-28 to Ser-42.
831702	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1359 as residues: Gly-1 to Gly-12, Glu-23 to Gly-28, Gln-56 to Trp-62, Lys-75 to Thr-103, Arg-217 to Asp-223.
832488	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1361 as residues: Leu-52 to Thr-59, Pro-86 to Ser-92, Arg-107 to Gly-118, Lys-121 to Gly-128.
833207	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1362 as residues:

	Val-29 to Arg-43, Glv-66 to Arg-75, Ser-94 to Glv-99, Ser-106 to Ser-112, Asp-135 to Leu-151.
835940	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1363 as residues: Arg-9 to Gln-35, Arg-94 to Cys-104.
837105	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1365 as residues: Ser-59 to Ser-65, Gln-75 to Gln-80.
837373	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1367 as residues: Arg-48 to Tyr-58, Asp-67 to Lys-75.
837687	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1368 as residues: Gly-1 to Asp-9, Ser-40 to Lys-46, Ser-65 to Pro-72, Lys-124 to Asn-137.
837991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1369 as residues: Lys-41 to Lys-48.
838442	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1370 as residues: Cys-7 to Glu-13, Tyr-27 to Phe-37, Phe-64 to Gly-72, Val-96 to Asp-105, Asp-111 to Ala-117, Arg-119 to Gly-125.
840541	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1371 as residues: Phe-38 to His-43, Asp-53 to Asp-61.
840543	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1372 as residues: Ala-26 to Pro-32, Ser-49 to Ala-59, Glu-106 to Arg-112, Gly-140 to Arg-149, Ala-159 to Trp-181, Glu-216 to Leu-229, Ile-243 to Ser-250, Phe-254 to Lys-259.
840563	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1374 as residues: Ala-67 to Pro-87.
840565	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1375 as residues: Gln-6 to Asn-13, Ser-29 to Lys-37, Arg-73 to Val-78.
840569	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1376 as residues: Ile-1 to Thr-6.
840570	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1377 as residues: Pro-9 to Asp-23.
840571	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1378 as residues: Gly-1 to Leu-6, Gln-13 to Ser-19.
840573	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1379 as residues: Arg-1 to Ala-7, Cys-16 to Cys-21, Arg-28 to Trp-33, Ala-36 to Gln-42, Arg-50 to Val-55, Gly-63 to Gly-74, Glu-100 to Lys-112, Lys-121 to Gln-126, Asp-132 to Leu-148, Ser-155 to Ser-161, Thr-167 to Ser-187, Arg-219 to Leu-228.
840574	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1380 as residues: Lys-60 to Lys-72, Asn-81 to Pro-88.
840575	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1381 as residues: Pro-1 to Arg-6, Tyr-16 to Gly-32, Ser-67 to Gly-74, Ser-95 to Gly-101, Glu-194 to Lys-218, Lys-295 to Leu-305, Met-332 to Glu-337, Leu-339 to Ala-347, Glu-353 to Leu-358, Ile-369 to Glu-375, Glu-437 to Gln-444, Glu-467 to Gly-478, Gly-481 to Gly-505.
840579	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1382 as residues: Pro-40 to Ala-50, Lys-71 to Leu-76, Glu-125 to Lys-138, Cys-153 to Ser-159, Arg-167 to Glu-173, Lys-210 to Ser-215, Asn-251 to Ser-260, Trp-289 to Ser-296, Ala-358 to Ala-363, Thr-369 to Gly-376, Asn-404 to Gly-410, Pro-425 to Glu-433, His-439 to Glu-450, Gln-470 to Ile-476, Thr-493 to Leu-499.
840580	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1383 as residues: Glu-13 to Ile-28, Pro-70 to Gly-75.
840581	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1384 as residues: Ser-1 to Gly-12, Thr-27 to Pro-36, Ser-50 to Met-56.
840605	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1385 as residues: Leu-12 to Leu-17, Glu-49 to Ser-54.
840610	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1388 as residues: Thr-19 to Lys-26, Gly-46 to Thr-52, Thr-63 to Glu-68, Gly-145 to Gly-153, Ser-236 to Thr-241, Ser-253 to Arg-263, Glu-291 to Asp-296.
840612	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1390 as residues: Arg-101 to Arg-108, Trp-119 to Ala-125, Ala-131 to Asn-138, Leu-142 to Thr-150, His-354 to Ile-370.

840622	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1392 as residues: Asp-6 to Gly-11, Ala-13 to Ser-28, His-40 to Thr-232, Arg-242 to Gly-247, Gly-268 to Gln-276.
840624	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1394 as residues: Lys-5 to Gly-12, Ala-20 to Met-26, Gly-49 to Ser-55, Pro-57 to Tyr-63.
840631	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1395 as residues: Glu-8 to Arg-24, Ser-36 to Ser-44, Phe-78 to Arg-84, Ser-116 to Trp-123, Gly-266 to Gly-274, Lys-327 to Lys-332.
840633	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1397 as residues: Ser-137 to Ala-146, Gln-165 to Gln-171.
840636	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1400 as residues: Lys-24 to Tyr-32, Tyr-42 to Lys-47, Gly-60 to Ala-66, Pro-68 to His-77.
840637	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1401 as residues: Ala-10 to Gln-16, Gly-29 to Glu-40, Arg-45 to Ser-51, Thr-62 to Pro-67.
840639	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1402 as residues: Pro-35 to Asn-48, Ser-66 to Ser-73, Asp-76 to Gly-81, Gly-115 to Glu-120, Asp-131 to Gly-147, Ser-152 to Gly-158, Pro-175 to Ser-184, Arg-206 to Asn-220.
840640	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1403 as residues: Ser-118 to Ile-123.
840650	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1404 as residues: Leu-30 to Gly-44, Gly-52 to Ala-57, Tyr-133 to Leu-140, Asp-207 to Ser-219, Gly-272 to Asn-281.
840652	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1405 as residues: Trp-33 to Gly-64.
840653	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1406 as residues: Pro-1 to Ser-6, Leu-14 to Ser-40, Leu-81 to Asp-93, Pro-125 to Phe-130, Gly-137 to Glu-148, Trp-238 to Arg-246, Gln-279 to Asp-295, Cys-305 to Pro-311.
840655	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1407 as residues: Pro-2 to His-7.
840659	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1408 as residues: Gln-1 to Val-15, Ser-21 to Gly-27, Pro-32 to Trp-42, Asn-272 to Arg-277, Pro-314 to Gln-336.
840660	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1409 as residues: Glu-1 to Asn-17.
840661	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1410 as residues: Cys-7 to Ser-20, Pro-35 to Pro-42, Pro-67 to Ile-80, Thr-94 to Met-100, Leu-122 to Cys-129.
840662	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1411 as residues: Gln-97 to Leu-102, Ala-130 to Ser-136, Ser-142 to Thr-148, Ala-180 to Ser-186, Pro-191 to Glu-198, Asn-234 to Leu-240, Ser-270 to His-280.
840663	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1412 as residues: Pro-1 to Gly-12.
840670	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1413 as residues: Gly-65 to Cys-71, Lys-81 to Gln-88, Thr-97 to Asp-106, Glu-135 to Gly-143, Pro-161 to Ala-169.
840671	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1414 as residues: Pro-4 to Thr-11, Ala-15 to Pro-20.
840672	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1415 as residues: Asp-3 to Ala-10, Val-23 to Thr-34, Gln-96 to Asp-101, Thr-118 to Gly-126, Ala-130 to Lys-140, Thr-156 to Ser-176, Pro-268 to Gln-275, Pro-296 to Gly-304, Pro-342 to Pro-348, Glu-382 to Asp-389, Met-408 to Glu-414, Pro-425 to Gln-443, Pro-457 to Tyr-478, Glu-481 to Tyr-505, Gly-514 to Arg-521, Pro-525 to Gly-547, Ala-555 to Gln-567.
840673	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1416 as residues: Ser-9 to Gly-15, Ser-57 to Arg-72, Lys-90 to Pro-111, Pro-138 to Ser-151, Asp-188 to Arg-193.
840677	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1418 as residues: Gly-17 to Asn-22, Ser-59 to Val-74, Glu-83 to Glu-89, Leu-91 to Ser-97, Glu-165 to Leu-183, Ala-197 to Ile-202, Ala-207 to Pro-212, Lys-227 to Lys-243, Pro-251 to His-258.
840678	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1419 as residues: Glu-43 to Glu-48, Gly-75 to Asp-81, Arg-92 to Ser-100, Asp-108 to Tyr-114, Ala-154 to Asn-161, Thr-266 to Gln-272.

840680	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1420 as residues: Pro-2 to Gly-8.
840691	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1421 as residues: Gln-58 to Ser-64, Asp-83 to Met-88, Ser-104 to Pro-114, Asn-137 to Ser-146, Pro-179 to Gly-185, Arg-206 to Glu-228, Gly-237 to Thr-258, Gln-269 to Asp-275.
840700	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1422 as residues: His-25 to Cys-32, Arg-46 to Glu-52.
840701	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1423 as residues: Gln-8 to Trp-13, Lys-21 to Asp-28, Ile-107 to Leu-112, Lys-125 to Trp-130, Leu-159 to Thr-164.
840702	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1424 as residues: Asp-22 to Met-37.
840705	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1425 as residues: Asp-4 to Pro-12, His-29 to Ala-39, Leu-43 to Glu-66, Asp-71 to Glu-78, Leu-84 to Asp-98, Glu-102 to Ile-121, Pro-137 to Tyr-143.
840715	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1426 as residues: Cys-1 to Gln-42.
840717	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1427 as residues: Cys-1 to Gln-6, Val-19 to Ala-24.
840718	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1428 as residues: Gln-1 to Ser-14.
840724	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1430 as residues: Cys-53 to Lys-59, Thr-61 to Cys-67, Gly-86 to Cys-93.
840725	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1431 as residues: Trp-22 to Thr-27.
840727	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1432 as residues: Thr-1 to Gln-8, Val-23 to Gln-28, Glu-51 to His-63, Glu-73 to Gln-91.
840731	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1433 as residues: Thr-35 to Glu-43, Leu-54 to Leu-60, Pro-89 to Gly-107, Val-109 to Gly-117, Gln-119 to Thr-125.
840733	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1434 as residues: Asp-33 to Ser-48, Pro-62 to Gly-76, Ser-80 to Gln-89, Gly-96 to Trp-109.
840734	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1435 as residues: Gln-12 to Gln-17.
840736	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1436 as residues: Arg-7 to Val-13, Leu-28 to Arg-33, Ser-69 to Gln-76.
840746	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1439 as residues: Asp-7 to Ser-13, Gln-21 to Lys-30, Gln-34 to Val-49, Glu-68 to Glu-73, Leu-79 to Leu-96, Glu-109 to Glu-115, Leu-146 to Ser-153, Leu-197 to Asn-206, Ser-218 to Glu-223, Ala-278 to Trp-283, Lys-297 to Phe-303, Ser-318 to Val-323.
840748	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1440 as residues: Lys-11 to Trp-24, Arg-30 to Ser-36, Arg-41 to Ser-55, Ser-68 to Arg-74, Leu-102 to Lys-108, Val-162 to Thr-167, Ser-188 to Lys-195, Glu-211 to His-216, Arg-253 to Arg-268, Ser-273 to Pro-279, Arg-325 to Glu-330, Lys-358 to Asp-364.
840750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1441 as residues: Met-48 to Gln-55, Ile-64 to Arg-69.
840751	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1442 as residues: Thr-30 to Lys-37, Gln-51 to Pro-56, Thr-58 to Val-72, Lys-81 to Val-88, Glu-90 to Asp-101, Gly-107 to Pro-113, Glu-115 to Ser-120, Lys-133 to Pro-143, Gly-172 to Asn-194, Val-196 to Gly-216, Phe-221 to Gln-226, Asn-255 to Lys-260, Leu-282 to Lys-290.
840757	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1443 as residues: Arg-8 to Gln-19, Arg-25 to Lys-38, Pro-91 to Pro-97.
840760	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1445 as residues: Gly-9 to Thr-14, Tyr-23 to Asp-32, Pro-40 to Pro-46.
840781	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1447 as residues: Glu-8 to Ser-13, Ser-26 to Lys-33, Lys-45 to Ser-50, Glu-81 to Glu-92, Asn-109 to Asp-115.
840789	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1448 as residues: Val-141 to Glu-147, Met-160 to Phe-166, Ser-176 to Asn-183, Arg-203 to Arg-210.

840790	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1449 as residues: Pro-17 to Asn-25.
840791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1450 as residues: Ser-62 to Gln-126, Ala-143 to Glu-182.
840798	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1451 as residues: Ser-87 to Gln-95.
840802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1452 as residues: Pro-22 to Glu-30, Lys-73 to Gly-79, Met-133 to Lys-140, Arg-166 to Lys-176.
840803	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1453 as residues: Ala-3 to Pro-12, Gln-29 to Ile-39, Ser-54 to Glu-72, Glu-79 to Asp-86, Pro-140 to Asp-147, Lys-161 to Lys-184, Val-188 to Thr-195, Asp-203 to Glu-215, Gln-231 to Phe-248, Gly-278 to Thr-281, Ser-290 to Asp-302, Gly-322 to Ser-336, Pro-342 to Leu-347, Lys-370 to Arg-394, Ser-424 to Ser-431, Asp-467 to Gln-483, Lys-507 to Ser-519, Phe-522 to Ser-567, Leu-578 to Gly-583, Thr-593 to Gln-600.
840811	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1455 as residues: Ser-10 to Gln-25, Pro-108 to Lys-124.
840814	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1457 as residues: Gln-29 to Arg-36.
840825	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1459 as residues: Ala-1 to Arg-10.
840827	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1461 as residues: Gly-13 to Gly-18, Pro-34 to Thr-45, Ser-47 to Asp-56, Ser-61 to Ser-73, Gly-81 to Gly-89, Gly-96 to Arg-102, Asp-118 to Glu-123, Thr-126 to Ala-132, Glu-178 to Glu-184, Glu-234 to Gly-260.
840828	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1462 as residues: Trp-53 to Asn-59, Thr-106 to Thr-111.
840829	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1463 as residues: Pro-16 to Thr-23, Val-67 to Asn-73.
840831	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1464 as residues: Thr-34 to Leu-42, Pro-82 to Tyr-88.
840837	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1466 as residues: Phe-39 to Ala-44, Lys-67 to Gln-77.
840838	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1467 as residues: Arg-2 to Gly-9, Arg-38 to Lys-46, Ser-53 to Ser-73, Asp-79 to Ala-84, Leu-129 to Glu-136, Glu-202 to Arg-210, Glu-216 to Ala-231, Glu-234 to Glu-254, Lys-259 to Leu-265.
840842	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1469 as residues: Phe-20 to Gly-25, Pro-73 to His-81, Pro-84 to Gly-90, Ser-94 to Arg-100.
840843	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1470 as residues: Gln-45 to Arg-55, Gly-74 to Leu-79, Lys-97 to Lys-103, Arg-108 to Lys-114, Asp-124 to Asp-138, His-153 to Gly-174, Lys-205 to Ala-223, Glu-230 to Arg-241, Glu-249 to Arg-256.
840845	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1471 as residues: Pro-29 to Trp-37, Pro-39 to Arg-44, Thr-51 to Trp-56, Ala-63 to Pro-73.
840851	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1473 as residues: Thr-23 to Glu-30, Gly-34 to Pro-51, Ser-53 to Pro-65, Lys-68 to Asp-85, Gly-97 to Gly-105, Ser-150 to Leu-163, Gln-205 to Thr-216, Thr-221 to Ser-227, Pro-237 to Leu-242, Val-258 to Asn-269, Glu-280 to Phe-291, Gly-295 to Pro-302, Gly-324 to Pro-332, Ser-342 to Ala-353, Arg-388 to Thr-426, Ser-432 to Tyr-439, Ala-452 to Gly-510, Glu-512 to Pro-524.
840854	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1475 as residues: Met-37 to Arg-43.
840858	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1476 as residues: Glu-37 to Lys-51, Thr-85 to Gly-91, Ser-115 to Trp-121, Tyr-177 to Asn-186.
840859	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1477 as residues: Asp-1 to Gln-7, Met-27 to Val-34.
840863	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1478 as residues: Lys-41 to Ala-51.
840868	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1479 as residues: Ala-3 to Trp-16, Lys-63 to Asn-72, Gln-112 to Leu-121, Leu-153 to Asp-159, Ala-163 to Leu-

	168, His-180 to Asp-187, Asp-347 to Gly-352, Met-356 to Ser-364, Pro-390 to Lys-401, Ala-519 to Thr-541, Arg-549 to Lys-554.
840869	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1480 as residues: Pro-6 to Asp-12, Arg-28 to Thr-37, Ile-50 to Lys-59, Ala-63 to Gly-70, Pro-89 to Tyr-96, Ser-103 to Ile-111, Thr-114 to Phe-121, Asp-141 to Pro-147, Arg-162 to Thr-172.
840870	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1481 as residues: Pro-18 to Gly-24.
840875	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1482 as residues: Thr-29 to Asn-37, Val-58 to Thr-63, Glu-114 to Glu-120, Thr-177 to Leu-184, Leu-196 to Ser-205.
840876	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1483 as residues: Glu-2 to Thr-7, Phe-119 to Trp-125, Thr-141 to Cys-147, Asn-210 to Gly-216, Thr-248 to Val-255, Pro-291 to Arg-296, Asp-308 to Asp-316, Glu-327 to Lys-335, Ser-341 to Thr-346.
840881	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1484 as residues: Asp-1 to Pro-14, Met-24 to Val-42, Lys-44 to Ser-60, Tyr-107 to Thr-114.
840883	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1485 as residues: Pro-28 to Cys-35, Glu-37 to Gln-43, Arg-51 to Arg-58, Gly-79 to Gly-85.
840886	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1486 as residues: Arg-1 to Ser-6, Gln-45 to Gln-51.
840887	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1487 as residues: Asn-77 to Met-83.
840891	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1488 as residues: Gln-1 to His-8, Arg-16 to Gln-25, Thr-32 to Ser-42.
840892	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1489 as residues: Pro-19 to Val-29, Lys-31 to Tyr-48.
840894	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1490 as residues: Pro-48 to Leu-55, Ser-65 to Gly-70, His-93 to His-126, Ile-128 to Glu-146, Leu-151 to Trp-159, Trp-161 to Pro-170, His-177 to Ala-182.
840896	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1491 as residues: Thr-37 to Ser-51.
840897	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1492 as residues: Ser-8 to Gly-13, Cys-32 to Ser-39, Cys-59 to Gly-64, Arg-72 to Gly-78, Leu-91 to Glu-104, Gly-118 to Glu-123, Asn-140 to Gln-149, Leu-157 to Ile-173, Glu-188 to Gln-209, Asn-222 to Lys-244, Gln-294 to Ile-300, Glu-336 to Val-342, Leu-346 to Lys-355.
840898	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1493 as residues: Ala-1 to Thr-6.
840904	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1494 as residues: Arg-7 to Gly-18, Asn-33 to Trp-40, Leu-48 to Thr-54, Pro-101 to Ala-106, Lys-119 to Val-126, Lys-169 to Leu-175, Gln-205 to Asp-216, Met-232 to Val-239, Arg-241 to Glu-252, Glu-260 to Pro-276, Ser-284 to Ile-291.
840905	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1495 as residues: Pro-17 to Ala-29, Leu-57 to His-67, Tyr-131 to Gly-137, Val-148 to Ser-153, Leu-214 to Gln-225, Ser-242 to Ser-247, Gly-261 to Ser-267, Arg-281 to Pro-286, Thr-299 to Lys-304, Ile-314 to Val-320, Lys-348 to Thr-366.
840908	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1496 as residues: Phe-49 to Glu-58, Leu-71 to Pro-85, Gln-105 to Leu-110, Thr-153 to Glu-158, Glu-168 to Ser-173, Asn-192 to Lys-197, Gln-207 to Asn-264, Pro-292 to Lys-299, Gln-331 to Leu-337, Ser-355 to Gly-362, Asp-381 to Gly-387, Val-396 to Asp-403, Thr-411 to His-416, Arg-451 to Gly-457, Glu-464 to Ala-469, Asn-492 to Gly-509, Tyr-518 to Thr-526, Glu-562 to Ser-567.
840909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1497 as residues: Pro-15 to Gly-29, Arg-34 to Ser-52.
840910	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1498 as residues: Arg-26 to Met-31.
840912	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1499 as residues: Ala-14 to His-19, Gln-31 to Thr-39, Phe-55 to Cys-60.
840916	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1500 as residues:

	Gly-7 to Leu-13.
840917	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1501 as residues: Ile-20 to Cys-26.
840918	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1502 as residues: Glu-59 to Thr-69, Thr-89 to Glu-96, Met-103 to Thr-110, Tyr-168 to Lys-176, Asn-196 to Ile-201, Thr-226 to Phe-235, Asp-244 to Glu-252, Lys-282 to Ser-290, Thr-325 to Thr-339, Lys-357 to Lys-362, Asn-397 to Tyr-403.
840922	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1503 as residues: Phe-1 to Lys-7.
840927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1505 as residues: Cys-32 to Lys-57.
840928	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1506 as residues: Arg-2 to Thr-7, Glu-65 to Trp-73, Glu-103 to Glu-110, Glu-219 to Asn-227, Glu-309 to Trp-320, Asp-389 to Asp-394.
840929	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1507 as residues: Pro-1 to Arg-7, Asp-21 to Lys-43, Lys-48 to Arg-53, Gln-59 to Gln-75, Pro-81 to Ala-86, Asp-127 to Lys-143, Glu-191 to Arg-197.
840930	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1508 as residues: Phe-1 to Cys-8, Ala-10 to Gly-23, Gln-114 to Lys-120, Glu-129 to Phe-135, Ile-155 to Gln-160, Ser-193 to Thr-199, Asp-214 to Gly-226, Asp-236 to Gly-245, Ala-283 to Arg-288, Ala-322 to Asp-331.
840931	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1509 as residues: Leu-28 to Asp-35, Leu-59 to Ser-65, Glu-111 to Lys-117, Gln-131 to Ala-137.
840941	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1510 as residues: Pro-16 to Ser-26, Arg-41 to Gly-49, Glu-51 to Arg-64, Tyr-69 to Phe-77, Thr-82 to Asp-90, Asp-168 to Gln-173, Lys-240 to Tyr-248.
840944	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1511 as residues: Gln-1 to Asp-10, Pro-104 to Glu-113, Pro-136 to Ala-142, Asn-152 to Lys-161.
840948	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1513 as residues: Ala-21 to His-26, Pro-41 to Gln-46, Lys-56 to Glu-66.
840953	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1515 as residues: Gly-1 to Ser-8, Arg-10 to Ser-15, Leu-17 to Gly-22, Lys-115 to Ala-130, Tyr-149 to Gly-156, Asn-181 to Glu-190, Glu-252 to Glu-257, Ser-339 to Asp-347, Leu-356 to Leu-361, Ser-387 to Lys-395, Thr-470 to Ile-476.
840954	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1516 as residues: Pro-12 to Phe-17, Asn-40 to Lys-55, Ser-105 to Thr-112, Lys-154 to Trp-168, Arg-176 to Phe-184, Leu-216 to Thr-224, Leu-237 to Val-242, Ala-365 to Val-370, Pro-379 to Gly-386, Leu-424 to Gly-430, Tyr-439 to Ser-451, Lys-459 to Tyr-464, Arg-595 to Asn-606, Asp-613 to Asn-621.
840958	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1517 as residues: Ala-1 to Lys-14, Glu-18 to Lys-40, Pro-61 to Thr-68, Pro-70 to Gln-78, Tyr-82 to Gly-90.
840960	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1518 as residues: Pro-42 to Asp-47, Thr-53 to Pro-59.
840968	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1519 as residues: Gln-5 to Glu-11.
840969	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1520 as residues: Glu-40 to His-45, Tyr-59 to Gly-68, Pro-107 to Pro-112, Leu-116 to Thr-121, Asp-139 to Lys-152.
840978	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1524 as residues: Ile-14 to Asp-19.
840980	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1525 as residues: Leu-1 to Pro-9, Val-13 to Val-41, Glu-79 to Met-86, Gln-89 to Lys-97, Glu-116 to Lys-128, Ser-130 to Gln-136, Arg-152 to Gly-158, Cys-161 to Lys-171, Pro-173 to Ala-182, Cys-184 to Ala-190, Leu-200 to Ser-206, Pro-225 to Leu-252.
840982	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1526 as residues: Pro-1 to Cys-9, Lys-27 to Ser-32, Glu-58 to Val-63, Ser-78 to Val-83.
840985	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1527 as residues:

	Asn-6 to Leu-17, Met-23 to Asp-33, His-56 to Gln-69, Arg-82 to Asp-89, Arg-92 to Lys-97, Ala-99 to Arg-104, Glu-140 to Asp-146, Ser-173 to Tyr-178, Cys-189 to Leu-194, Val-239 to Asn-245, Glu-266 to Arg-276.
840989	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1528 as residues: Asn-72 to Ile-78, Gly-102 to Asp-109, Arg-150 to Trp-158, Phe-255 to Pro-266, Glu-272 to Lys-277.
840991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1529 as residues: Thr-10 to Ala-17, His-24 to Leu-30, Ala-128 to Val-136.
840996	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1530 as residues: Cys-107 to Gln-112, Lys-142 to Ser-148.
840997	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1531 as residues: Ile-25 to Pro-35, Asp-37 to Thr-42, Ala-56 to Phe-71, Arg-75 to Gln-82, Thr-127 to Tyr-139.
840998	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1532 as residues: Lys-19 to Thr-24, Pro-35 to Gln-130.
840999	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1533 as residues: Phe-44 to Arg-53.
841000	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1534 as residues: Ala-4 to Pro-13.
841002	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1535 as residues: Pro-8 to Ser-18, His-27 to Ser-39, Pro-50 to Gly-59.
841003	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1536 as residues: Pro-24 to Glu-31.
841008	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1537 as residues: Cys-10 to Cys-16, Thr-114 to Gly-120, Asn-200 to Lys-209.
841013	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1538 as residues: Phe-58 to Asn-66, Ala-82 to Gln-88, Ser-169 to Glu-178, Pro-222 to Gly-227, Glu-283 to Glu-289, Ala-314 to Gly-321, Ile-370 to Asn-376, Lys-409 to Ala-423, Asp-444 to Arg-449, Ser-456 to Glu-463, Asn-472 to Asn-477.
841014	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1539 as residues: Asn-8 to Phe-17, Gly-58 to Asp-64, Glu-186 to Ser-191, Ala-266 to Ile-271, Thr-300 to Lys-309, Val-327 to Met-332.
841015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1540 as residues: Tyr-17 to Thr-29, Lys-35 to Glu-40.
841019	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1542 as residues: Phe-9 to Phe-16.
841024	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1543 as residues: Ser-6 to Gly-15, Ala-90 to Gly-96, Val-119 to Trp-127, Val-147 to Lys-155, Ala-174 to Glu-181, Ala-231 to Leu-239.
841025	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1544 as residues: Leu-18 to His-27, Asp-29 to Ser-42, Glu-62 to Asn-72, Ser-76 to Glu-81.
841026	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1545 as residues: Ala-3 to Gly-10, Lys-41 to Gly-48, Pro-69 to Ser-81, Pro-92 to Thr-97, Asn-101 to Lys-110, Gly-173 to Gly-182, Arg-188 to Asn-199.
841027	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1546 as residues: Pro-1 to Arg-19, Asp-42 to Glu-48, Asp-70 to Tyr-79, Asn-81 to Gly-88, Ala-91 to Gly-98, Glu-153 to Pro-163.
841029	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1547 as residues: Arg-50 to Ser-58, Arg-66 to Asp-73, Pro-96 to Ser-102, Gln-133 to Arg-142.
841030	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1548 as residues: Ser-23 to Gln-30.
841034	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1550 as residues: Ser-56 to Lys-61.
841036	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1551 as residues: Leu-89 to Lys-102.
841039	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1552 as residues: Glu-19 to Ser-24, Ser-52 to Gly-60, Ser-67 to Gly-74, Lys-142 to Gly-148, Pro-178 to Arg-184.

841048	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1554 as residues: Met-22 to Tyr-49, Arg-60 to Thr-69, Gln-93 to Glu-111, Pro-113 to Glu-139, His-152 to Ser-162, Lys-172 to Glu-178, Ser-183 to Ile-188, Asn-191 to Arg-201, Arg-251 to Asn-259, Thr-297 to Arg-303, Val-379 to Gln-401, Ser-407 to Pro-414, Thr-428 to Lys-446.
841050	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1556 as residues: Ile-6 to Asn-15.
841052	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1557 as residues: Pro-37 to Arg-42, Asn-83 to Phe-90, Lys-187 to Cys-192, Asp-209 to Gly-215, His-236 to Lys-243, Tyr-263 to Gly-276, Thr-308 to Gly-314, Glu-346 to Asp-351.
841054	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1558 as residues: Pro-8 to Glu-18, Ala-47 to Gly-53.
841055	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1559 as residues: Val-13 to Leu-31.
841056	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1560 as residues: Arg-8 to Phe-13, Arg-29 to Val-36.
841060	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1561 as residues: Asp-69 to Gln-74.
841062	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1563 as residues: Gly-1 to Lys-6, Thr-10 to Lys-16, Asp-22 to Pro-35, Pro-62 to Asp-77, Ile-85 to Met-97, Leu-130 to Thr-135, Lys-206 to Gly-213, Leu-234 to Ser-242, Leu-334 to Glu-341, Ser-354 to Lys-369, Glu-398 to Lys-409, Glu-425 to Glu-477.
841063	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1564 as residues: Ala-1 to Trp-12, Glu-49 to Gly-56, Lys-99 to Thr-110, Glu-147 to Lys-154.
841067	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1565 as residues: Ser-7 to Ala-12, Gly-14 to Met-30, Lys-52 to Ala-58.
841074	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1566 as residues: Ala-1 to Gln-6, Glu-22 to Arg-30, Leu-43 to Ser-52, Glu-61 to Lys-70, Lys-75 to Glu-84, Thr-105 to Lys-110, Asp-131 to Ala-143, Ser-151 to Thr-158, Thr-200 to Asp-208.
841076	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1567 as residues: Lys-1 to Gly-6, Asp-13 to Glu-27.
841083	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1569 as residues: Leu-42 to Lys-49, Glu-63 to Ser-68, Glu-93 to Gln-98, Asn-109 to Ser-115, Met-147 to Lys-152.
841093	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1571 as residues: Pro-5 to Glu-14, Ala-84 to His-90, Thr-93 to Gly-99, Asn-124 to Val-133, Met-144 to Val-149, Thr-192 to Glu-200.
841097	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1572 as residues: Pro-46 to Glu-56, Phe-65 to Ser-73, Glu-114 to Asp-121, Thr-132 to Gln-139, Asp-171 to Pro-177, Thr-215 to Val-221.
841098	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1573 as residues: Arg-9 to Gly-14, Met-36 to Lys-57, Pro-93 to Gly-98.
841113	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1575 as residues: Gln-10 to Gly-18.
841115	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1576 as residues: Ile-1 to Lys-13, Thr-36 to Ala-42, Asn-49 to Leu-55, Phe-59 to Arg-70, Asp-80 to Arg-86, Lys-92 to Lys-98.
841117	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1578 as residues: Arg-1 to Glu-26, Thr-59 to Glu-64, Gln-69 to Met-77, Arg-79 to Ser-84, Pro-86 to Pro-97, Arg-104 to Lys-121, Ala-133 to Arg-141, Leu-162 to Ser-169.
841127	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1580 as residues: Pro-1 to Pro-12, Arg-51 to Ile-56, Lys-69 to Arg-85, Glu-115 to Arg-122, Gly-129 to Gln-134, Lys-138 to Lys-156, Gly-163 to Pro-170.
841128	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1581 as residues: Pro-75 to Glu-91, Glu-121 to Gly-126, Ile-149 to Lys-155, Ala-185 to Asp-201, Glu-237 to Gly-252, Leu-256 to Ser-276.
841134	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1584 as residues: Lys-43 to Leu-48, Lys-54 to Ala-62, Asn-75 to Ala-82, Glu-135 to Asp-140, Glu-173 to Leu-178.

	Lys-213 to Tyr-222.
841138	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1587 as residues: Arg-68 to Gln-74, Ser-85 to Asp-115, Arg-133 to Lys-144, Arg-152 to Ile-165, Pro-184 to Lys-191, Leu-198 to Lys-215, Val-235 to Glu-240, Asp-246 to Asn-266, Glu-284 to Pro-292.
841141	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1589 as residues: Pro-16 to Glu-27, Pro-36 to Phe-43, Asn-71 to Ser-84, Thr-107 to Ser-115, Glu-147 to Lys-161, Pro-167 to Ser-185, Ser-187 to Ser-206.
841145	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1591 as residues: Glu-33 to Pro-40, Arg-48 to Pro-56, Met-71 to Gly-76, Ser-103 to Arg-115.
841146	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1592 as residues: Lys-21 to Thr-26, Thr-37 to Pro-42.
841150	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1593 as residues: Ser-56 to Thr-62.
841153	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1594 as residues: Glu-4 to Trp-9.
841154	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1595 as residues: Asp-24 to Tyr-29, Ser-34 to Asn-42, Leu-45 to Lys-61, Thr-117 to Ser-124, Lys-153 to Asp-158, Glu-174 to Lys-180, Leu-188 to Gly-204, Ala-220 to Leu-227, Gly-262 to His-268, Lys-276 to Thr-287, Phe-307 to Pro-319, Thr-345 to Met-351, Gln-427 to Ala-432, Asp-438 to Gln-443.
841156	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1596 as residues: Glu-4 to Gly-12, Thr-21 to Gln-27, Pro-40 to Ser-47, Pro-50 to Ser-61, Val-101 to Cys-107, Lys-138 to Gly-147, Gln-150 to Tyr-156, Lys-169 to Thr-174.
841157	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1597 as residues: Val-35 to Ala-41, Gln-56 to Trp-70.
841159	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1598 as residues: Gln-1 to Arg-7, Arg-14 to Glu-22, Ala-43 to Asp-55, Thr-65 to Arg-71.
841164	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1599 as residues: Arg-1 to Cys-11, Arg-18 to Arg-25, Gly-83 to Glu-88, Gly-108 to Lys-113.
841167	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1600 as residues: Arg-16 to Asp-22.
841170	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1601 as residues: Ala-1 to Ala-14, Ala-37 to Asp-45, Thr-55 to Leu-62, Glu-76 to Gly-82, Ile-101 to Gly-110, Pro-119 to Gly-127, Pro-129 to Asp-142, Lys-196 to Ser-210, Pro-216 to Tyr-246.
841173	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1602 as residues: Arg-52 to Gln-57, Asp-181 to Gly-187, Ser-260 to Val-271, Lys-285 to Asp-290.
841178	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1604 as residues: Ser-1 to Ala-9, Ala-14 to Ile-30, Pro-41 to Ser-50, Asn-56 to Arg-63, Asp-95 to Lys-102, Pro-126 to Ser-132.
841181	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1606 as residues: Thr-3 to Arg-12.
841182	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1607 as residues: Gly-12 to Gln-26, Cys-34 to Gly-49, Glu-86 to Tyr-93, Phe-103 to Thr-139, Asp-145 to Gln-153, Tyr-167 to Arg-176, Ser-192 to Gly-200, Ala-219 to Gly-226, Glu-234 to Trp-242.
841187	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1609 as residues: Glu-1 to Gly-15, Pro-23 to Val-48, Pro-58 to Glu-63, Thr-79 to Trp-91, Asn-203 to Lys-213.
841188	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1610 as residues: Arg-1 to Gly-7, Ile-92 to Tyr-98, Arg-153 to Gly-159, Ala-319 to Ser-324, Lys-350 to Glu-359.
841189	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1611 as residues: Arg-13 to Ala-21, Thr-29 to Arg-34, Glu-41 to Ala-50, Ser-65 to Glu-71, Glu-108 to Glu-117, Ile-144 to Arg-154, Gly-159 to His-185, Lys-189 to Tyr-197.
841192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1612 as residues: Gly-56 to Leu-63, Gln-188 to Lys-193, His-200 to Gly-205, Leu-208 to Asn-215, Thr-358 to Lys-367, Lys-369 to Gln-377, His-426 to Arg-431, Tyr-437 to Glu-446, Glu-459 to Pro-476.
841194	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1613 as residues: Phe-54 to Ser-59, Thr-63 to Asp-69.
841195	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1614 as residues:

	His-1 to Gln-6, Ala-66 to Gly-79, Leu-88 to Asp-95, Glu-121 to Ile-126, Pro-140 to Pro-147, Ile-173 to Trp-180, Asn-195 to Tyr-206.
841198	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1615 as residues: Gln-29 to Arg-34, Thr-65 to Thr-76, Arg-100 to Arg-108, Leu-163 to Ala-173.
841201	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1617 as residues: Gln-3 to Lys-10, Pro-42 to Pro-50, Ser-66 to Ser-80, Glu-107 to Ala-121.
841202	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1618 as residues: Ser-11 to Trp-23, Glu-25 to Gly-32, Ala-56 to Gly-67, Glu-80 to Pro-96, Ala-166 to Leu-177, Asn-222 to His-231, Met-239 to Gly-249, Gly-318 to Pro-338.
841209	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1619 as residues: Arg-4 to Leu-27, Gln-63 to Leu-82, Pro-168 to Ser-175.
841213	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1621 as residues: Val-17 to Tyr-22, Cys-32 to Asp-49, Ser-104 to Pro-114.
841219	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1623 as residues: Leu-10 to Glu-28, Lys-54 to Gln-60.
841222	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1624 as residues: Ile-9 to Ser-14, Pro-68 to Cys-80, Ser-82 to Thr-87, Ile-136 to His-155, Lys-214 to Asn-224.
841223	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1625 as residues: Pro-12 to Glu-17.
841226	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1627 as residues: Ala-40 to Thr-52.
841227	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1628 as residues: Val-54 to Asn-60, Glu-81 to Thr-87, Asn-103 to Glu-108, Asn-163 to His-168, Ile-170 to Tyr-175.
841233	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1632 as residues: Gly-8 to Gly-20, Ser-81 to Phe-89, Leu-135 to Gln-140, Glu-156 to Tyr-168.
841234	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1633 as residues: Lys-65 to Phe-70, Asp-99 to Ile-104, Arg-122 to Asp-128, Leu-244 to Ile-250, Leu-258 to Leu-268, Ala-270 to Lys-286, Lys-310 to Asp-318, Asn-338 to Gln-344, Asp-360 to Leu-369, Lys-414 to Gln-422, Glu-435 to Arg-449, Lys-471 to Phe-476, Arg-498 to Leu-503, Ala-526 to Gly-534, Ala-536 to Pro-559, Pro-586 to Tyr-612, Tyr-624 to Tyr-629, Gln-639 to Gln-668.
841236	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1634 as residues: Lys-5 to Pro-18, Glu-24 to Ser-36, Pro-57 to Gly-63.
841239	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1636 as residues: Arg-1 to Ser-6.
841243	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1638 as residues: Gln-1 to Asp-7, Pro-26 to Ser-31, Leu-41 to Arg-46, Gly-57 to Thr-65, Lys-71 to Lys-76.
841248	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1639 as residues: Ala-8 to Thr-23, Pro-35 to Met-41, Asn-60 to Thr-65, Asn-89 to Gly-94, Pro-161 to Leu-167, Asp-184 to Trp-189, Phe-192 to Leu-206, Arg-215 to Leu-221.
841250	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1640 as residues: Asn-13 to Gly-22, Gln-24 to Lys-29, Ser-44 to Gly-51, Thr-128 to Asp-138, Glu-166 to Leu-175, Arg-187 to Ala-192, Pro-240 to Ala-256, Ser-259 to Trp-265, Met-281 to Lys-288, Leu-318 to Trp-356, Ser-379 to Thr-385, Phe-409 to Tyr-419.
841251	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1641 as residues: Arg-13 to Phe-20, His-22 to Ser-27, Gln-70 to Phe-76.
841254	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1642 as residues: Thr-1 to Lys-15, Gln-41 to Glu-46.
841263	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1643 as residues: Ser-27 to Arg-35, Leu-76 to Trp-85, Arg-112 to Thr-118.
841269	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1645 as residues: Lys-12 to Lys-19.
841273	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1647 as residues: Tyr-3 to Asn-9.
841277	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1649 as residues: Pro-55 to Ser-62, Arg-124 to Ile-129, Arg-145 to Asn-151, Asn-186 to Asn-196, Lys-267 to Lys-

	274, Arg-368 to Arg-373.
841278	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1650 as residues: Ala-6 to Pro-13, Asn-19 to Phe-24.
841279	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1651 as residues: Thr-3 to Gly-12, Arg-19 to Ala-24, Arg-30 to Gly-43, Pro-46 to Trp-51, Gly-77 to Arg-85.
841280	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1652 as residues: Ser-14 to Thr-20, Glu-44 to Gly-50, Lys-68 to Pro-76, Glu-91 to Glu-96, Ala-110 to Lys-116, Lys-124 to His-131, Gly-164 to Gln-173, Leu-191 to Asn-200, Met-215 to Ser-221, Gln-236 to Lys-258, Pro-266 to Asn-271, Pro-279 to Asp-286.
841282	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1653 as residues: Leu-3 to Lys-8.
841283	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1654 as residues: Tyr-1 to Glu-9, Ala-12 to Ser-18, His-63 to Phe-77, Asn-98 to Arg-110.
841286	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1655 as residues: Ser-13 to Arg-19, Leu-28 to Val-35, Pro-37 to Gly-57, Ser-81 to Pro-87, Ile-102 to Arg-111.
841287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1656 as residues: Arg-1 to Ala-10, Val-23 to Phe-42, Asp-60 to Tyr-69, Pro-71 to Ser-79.
841288	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1657 as residues: Ser-4 to Pro-9, Arg-18 to Pro-26.
841291	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1658 as residues: Lys-16 to Ser-23, Gln-56 to Asp-63, Lys-137 to His-145, Glu-149 to His-156, Glu-163 to Gly-171, Pro-173 to Ala-180, Lys-189 to Ala-206, Glu-208 to Gln-214, Pro-231 to Ser-240.
841294	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1660 as residues: Gly-6 to Gly-12, Glu-19 to Pro-37, Gly-43 to Pro-55, Asp-62 to Gln-78, Arg-89 to Gln-95, Lys-99 to Arg-118, Glu-123 to Ala-139.
841301	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1663 as residues: Asn-8 to Arg-13, Gly-36 to Leu-43, Arg-53 to Cys-59.
841303	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1664 as residues: Pro-23 to Gly-35, Pro-38 to Phe-45, Pro-47 to Gly-56, Val-68 to Tyr-73, Gly-123 to Gly-135, Met-150 to Gln-164, Arg-212 to Ile-220, Arg-284 to Ile-289, Tyr-296 to His-315, Gln-325 to Ile-334, Thr-471 to Arg-476.
841304	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1665 as residues: Phe-33 to Arg-47, Asn-65 to Gly-71, Asp-95 to Gly-100, Asp-152 to Asn-163, His-223 to Gly-229.
841305	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1666 as residues: Gly-5 to Trp-19, Pro-21 to Ser-35, Pro-42 to Ser-58, Pro-64 to Asp-75.
841309	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1667 as residues: Lys-1 to Lys-6, Lys-18 to Asp-25, Thr-46 to Arg-64, His-97 to Lys-105, Glu-113 to Ala-118, Asn-126 to Gly-137, Thr-142 to Pro-147, Glu-155 to Ile-173, Ala-175 to Asn-184, Ser-188 to Gly-222, Glu-228 to Ala-242, Ala-263 to Asp-272, Thr-277 to Asp-288, Lys-293 to Met-308, Ile-348 to Gly-359, Pro-361 to Thr-386, Pro-403 to Arg-411, Asp-466 to Gln-473, Arg-479 to Thr-493, Lys-507 to Lys-513.
841314	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1668 as residues: Leu-4 to Ala-11, Phe-106 to Trp-112, Lys-204 to Ile-209, Ser-224 to Leu-236, Pro-254 to Ser-262, Phe-282 to Met-295.
841316	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1669 as residues: Pro-60 to Ser-67, Lys-86 to Ile-92, Arg-125 to Lys-130, Glu-155 to Asp-161, Glu-170 to Ser-176, Thr-181 to Val-187, Leu-198 to Asn-203, Gln-258 to Lys-263, Pro-271 to Asn-276, Phe-286 to Glu-292.
841318	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1670 as residues: Pro-14 to Trp-25, His-36 to Arg-41, Gly-66 to Tyr-73, Glu-82 to Pro-89.
841321	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1671 as residues: Asp-11 to Gly-19, Asp-26 to Val-31, Ala-52 to Asn-71, Gly-75 to Gly-81, Pro-88 to Gly-119, Pro-125 to Pro-180, Gly-187 to Gly-193, Tyr-196 to Tyr-218.
841324	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1672 as residues: Gly-45 to Val-54, Trp-67 to Gly-75, Asp-82 to Asn-90, Ala-124 to Trp-132, Thr-139 to Gln-145.

841326	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1673 as residues: Thr-45 to Asn-50, Lys-60 to Arg-73, Arg-81 to Asp-87, Lys-91 to Ser-96, Pro-105 to Gly-114, Ser-130 to Leu-136, Leu-145 to Ile-154, Cys-279 to Pro-284, Thr-321 to Glu-326, Pro-389 to Thr-398, Ala-406 to Ile-412, Ala-431 to Glu-438, Lys-495 to Glu-500, Asn-520 to Val-526, Glu-541 to Asn-547, Thr-552 to Tyr-557.
841328	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1674 as residues: Asn-64 to Ala-78, Ser-155 to Ala-169, Lys-290 to Asp-314.
841329	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1675 as residues: Leu-10 to Trp-18, Arg-21 to Leu-32, Pro-35 to Leu-55, Arg-74 to Phe-90, Pro-106 to Trp-115, Val-142 to Thr-152.
841330	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1676 as residues: Gly-14 to Ala-19, Arg-34 to Arg-41.
841333	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1677 as residues: Leu-20 to Val-26.
841335	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1679 as residues: Asn-10 to Cys-17.
841336	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1680 as residues: Lys-1 to Arg-9, Ala-57 to Met-66, Ile-70 to Glu-78, Ile-104 to Gly-125, Thr-155 to Glu-160, Pro-174 to Leu-184, Ala-200 to Arg-206, Ser-231 to Ser-255, Glu-281 to Asp-287.
841337	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1681 as residues: Arg-79 to Val-86, Ala-111 to Glu-125, Pro-148 to Met-153, Arg-180 to Leu-188, Pro-275 to Gly-296, Pro-336 to Phe-350, Gly-353 to Ser-362, Val-364 to Arg-371.
841340	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1683 as residues: Pro-39 to Ser-46.
841341	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1684 as residues: Pro-9 to Gly-23, Glu-43 to Ala-51, Ser-62 to Gly-91.
841343	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1686 as residues: Lys-49 to Gly-66, Ala-78 to Ser-85, Gly-90 to Thr-97, Arg-124 to Gly-129.
841352	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1688 as residues: Arg-37 to Leu-47, Gln-93 to Asp-112, Arg-114 to Arg-119, Arg-124 to Arg-142.
841353	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1689 as residues: Leu-23 to Thr-28, Ile-47 to Lys-56, Arg-91 to Gln-99, Gly-111 to Ser-119.
841354	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1690 as residues: Ser-36 to Arg-42.
841360	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1691 as residues: Asn-1 to Thr-11, Pro-64 to Phe-75, Phe-117 to Ile-122, Glu-124 to Arg-131, Trp-142 to Gln-147, Thr-176 to Ser-185, Arg-208 to Gly-215, Gln-238 to Ser-244, Ala-246 to Val-256, Ser-264 to Lys-272.
841405	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1693 as residues: Leu-1 to Gly-14, Arg-21 to Glu-26, Lys-62 to Val-73, His-131 to Asp-136, Glu-142 to Tyr-158, Val-162 to Gly-169, Gln-183 to Gly-189, Glu-205 to Gly-210, Gln-222 to Asp-231, Gly-237 to Tyr-244, Ala-251 to Leu-267, Asp-298 to Asn-305, Glu-332 to Lys-337, Arg-344 to Ala-349.
841526	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1694 as residues: Pro-1 to Arg-8.
841712	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1695 as residues: Gln-34 to Lys-44, Ser-70 to Leu-75, Ala-79 to Pro-89, Glu-94 to Thr-101, Gln-103 to Ser-112.
842042	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1697 as residues: Arg-64 to Glu-69, Ile-78 to Tyr-86, Asp-128 to Gly-148, Pro-166 to Pro-187, Ala-194 to Lys-239, Ala-243 to Ala-255.
842453	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1698 as residues: Gly-41 to Gly-53, Gly-65 to Arg-74.
842635	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1699 as residues: Cys-2 to Asp-11, Lys-39 to Phe-55, Tyr-72 to Trp-78, Thr-154 to Lys-164, Ser-191 to Lys-203, Asp-218 to Asp-223.
842927	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1700 as residues: Pro-8 to Trp-14, Gly-33 to Glu-48, Arg-58 to Lys-67, Thr-76 to Gln-96, Ala-98 to Ser-118, Cys-

	193 to Thr-201, Leu-225 to Trp-232, Asp-256 to Phe-262.
843237	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1703 as residues: His-1 to Gly-14, Leu-36 to Ser-41, Gln-45 to Arg-59, Gly-66 to Arg-91, Lys-104 to Trp-113, Arg-143 to Leu-148, Val-172 to Val-181, Pro-235 to Lys-242.
843381	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1704 as residues: Arg-9 to Arg-14, Gly-27 to Cys-32, Ser-53 to Leu-61, Ala-66 to Phe-71.
843823	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1706 as residues: Asp-11 to Tyr-16.
844056	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1707 as residues: Lys-145 to Thr-159, Ser-167 to Lys-176, Asn-216 to Lys-224.
844344	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1709 as residues: Gly-4 to Asp-9, Glu-23 to Lys-31, Asn-38 to Tyr-47.
844368	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1710 as residues: His-5 to Gly-15, Pro-97 to Cys-103.
844408	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1711 as residues: Thr-49 to Gln-60.
844867	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1713 as residues: Ile-49 to Thr-60.
845281	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1715 as residues: Gly-5 to Arg-12.
845288	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1716 as residues: Ala-1 to Gly-6, Ala-8 to Val-15, Ala-159 to Pro-164.
845750	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1717 as residues: Arg-1 to Thr-9.
845809	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1718 as residues: Glu-9 to Arg-14, Thr-19 to Arg-27, Asp-48 to Ile-57, Gln-63 to Leu-75, Cys-89 to Thr-104, Gly-106 to Pro-113, Gly-127 to Thr-133, Arg-144 to Asn-157, Ile-179 to Arg-199.
846077	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1719 as residues: Pro-11 to Trp-18, Cys-59 to Pro-68, Thr-77 to Glu-86, Arg-94 to Asn-102.
HPRT105R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1721 as residues: Pro-22 to Tyr-34.
HPDED94R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1724 as residues: Gly-1 to Glu-6.
HDTGH11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1725 as residues: Thr-32 to Met-37.
HTEJR60R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1726 as residues: Ala-1 to Ser-6.
HAGGY86R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1727 as residues: Leu-25 to Trp-40, Val-49 to His-56, Leu-60 to Asn-67.
HP1AU47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1728 as residues: Glu-88 to Leu-93.
HCGAD89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1729 as residues: Glu-30 to Asp-45.
HAPOD39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1730 as residues: Tyr-21 to Ala-28, Ser-74 to Gly-81.
HDRAA14R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1733 as residues: Ala-1 to Pro-8, Ala-10 to Val-16, Pro-43 to Leu-52.
HSLCA48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1734 as residues: Glu-26 to Leu-31.
HMQDF20R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1736 as residues: Phe-33 to Ala-43, His-86 to Ser-93.
HCHOH06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1737 as residues: Gly-4 to Lys-10, Arg-17 to Glu-24, Gln-36 to Glu-41, Arg-61 to Arg-76.
HLDRN91R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1740 as residues: Arg-22 to Gln-27, Ser-33 to Val-38, Lys-46 to Gly-57, Gln-92 to Gly-97.
HE6GO78R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1743 as residues:

	Ser-3 to Trp-12.
HSYBY17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1745 as residues: Gln-30 to Pro-36.
HPJCS07R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1746 as residues: Tyr-25 to Phe-32.
HFKFH08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1748 as residues: Arg-2 to Gln-8, Val-49 to Asn-54, Gln-58 to Tyr-64.
HPIBI27R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1750 as residues: Glu-17 to Asp-22, Pro-46 to Arg-52, Pro-75 to Asp-84.
HSKJG37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1751 as residues: Leu-66 to Gly-72, Asp-89 to Pro-97, Thr-104 to Leu-110.
H2LAZ24R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1752 as residues: Pro-20 to Ala-26, Ser-107 to Ala-113, Asp-129 to Gly-135, Thr-139 to Asp-146, Ser-152 to Arg-168, Glu-173 to Pro-180.
H2LAS11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1756 as residues: Pro-20 to Ser-25, Lys-67 to Phe-76, Pro-78 to Asn-86, Asp-100 to Gly-108, Arg-116 to Gly-122, Glu-153 to Ala-158.
HADMC73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1758 as residues: Ala-1 to Tyr-9.
HDTDX66R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1760 as residues: Met-2 to Leu-9, Lys-11 to Pro-28, Asp-57 to Leu-68, Gln-81 to Ser-96, Ser-98 to Arg-106.
HL PBB39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1761 as residues: Cys-27 to Lys-33, Thr-35 to Cys-41.
HKABU38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1763 as residues: Pro-1 to Pro-11, Ala-17 to Lys-25, Asp-54 to Leu-59, Thr-66 to Arg-76, Arg-90 to Pro-107, Pro-139 to Glu-146.
HATAI03R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1765 as residues: Phe-1 to Asn-6.
HCEDE25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1766 as residues: Ala-6 to Thr-13.
H2LAO77R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1770 as residues: Ala-16 to Pro-30, Thr-44 to Val-57, Lys-75 to Gly-80, Asp-92 to Leu-102, Ala-113 to Tyr-120.
HNTRW15R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1771 as residues: Met-3 to Lys-9, Ala-16 to Trp-37.
HULBL38R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1773 as residues: Cys-1 to Glu-6, Asp-52 to Asp-65, Lys-82 to Pro-88.
HNTBK49R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1774 as residues: Pro-40 to Gly-45.
HBAFS48R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1775 as residues: Pro-1 to Glu-18, Pro-37 to Met-44.
HOHBU75R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1777 as residues: His-24 to Gly-29, Glu-32 to Asp-37, Gly-47 to Pro-60.
HSLBA61R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1779 as residues: Asn-37 to Thr-42.
HKAKR61R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1782 as residues: Arg-1 to Thr-7.
H2LAD40R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1785 as residues: Trp-13 to Asp-19, Cys-29 to Gln-34, Ala-41 to Arg-52, Gly-54 to Gln-59, Arg-69 to Pro-78.
H2MBU27R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1788 as residues: Asp-3 to Lys-9, Arg-88 to Gln-95.
HDSAHS3R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1789 as residues: Asp-7 to Lys-13.
HAIDF69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1790 as residues: Gln-13 to Pro-22.
HTWJC11R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1793 as residues: Pro-27 to Val-32.

HKAEC40R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1794 as residues: Lys-86 to Lys-91.
HCFNM70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1795 as residues: Thr-19 to Lys-24.
HKBAB93R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1796 as residues: Lys-9 to Tyr-26, Arg-48 to Lys-53, Ser-68 to Thr-75, Ala-84 to Leu-89.
HMAEA94R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1800 as residues: His-60 to Asp-69, Phe-87 to Ala-93.
HMWEA08R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1801 as residues: Met-3 to Thr-8, Tyr-33 to Gly-38, Lys-54 to Glu-65.
HRACC09R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1803 as residues: Lys-7 to Trp-18.
HOEEC67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1804 as residues: Lys-24 to Glu-31.
HPFEA40R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1805 as residues: Arg-4 to Ile-20.
HHECI89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1807 as residues: Ala-1 to Arg-12, Pro-22 to Met-28, Glu-53 to Thr-61, Gly-90 to Ile-97.
HSDFV03R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1808 as residues: Ser-18 to Phe-24, Pro-40 to Thr-46.
HTXPN01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1809 as residues: Lys-19 to Glu-28.
HACBH95R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1813 as residues: Pro-43 to Gly-51.
HACBY16R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1814 as residues: Arg-1 to Glu-16.
HAHAD34R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1816 as residues: Gly-13 to Ala-21.
HAJAN69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1817 as residues: Gly-1 to Gly-22, Pro-61 to Ala-70.
HAPPR17R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1819 as residues: Asn-8 to Met-13, Asp-15 to Met-21.
HBGBE20R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1824 as residues: Arg-28 to Leu-33.
HBMVT43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1828 as residues: Pro-1 to Asn-8.
HCFLN25R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1830 as residues: Gly-16 to Trp-21, Pro-24 to Leu-32.
HCQAW59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1831 as residues: Gly-1 to Gly-8, Pro-11 to Asn-21.
HDPMA46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1832 as residues: Gly-14 to Gly-32, Pro-61 to Gly-66.
HDTAQ26R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1833 as residues: Ser-1 to Gly-7.
HDTLD39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1835 as residues: Thr-14 to Ser-44.
HE2PO63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1836 as residues: Phe-11 to Lys-17, Gly-36 to Gly-43.
HELHK95R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1838 as residues: Pro-20 to Pro-28.
HETIB72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1840 as residues: Gln-1 to Glu-9.
HFIYH65R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1842 as residues: Ala-2 to His-8, Gly-26 to Cys-32.
HKIXO47R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1848 as residues: Ala-1 to Arg-8, Val-12 to Lys-25.

HLWBC80R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1851 as residues: Arg-72 to Gly-80, Leu-86 to Phe-92.
HLYAV50R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1852 as residues: Asp-1 to Gly-6, Gly-44 to Arg-50.
HMEKY67R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1853 as residues: Arg-12 to Phe-24, Pro-32 to Ser-43.
HOUDQ92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1858 as residues: Arg-1 to Cys-7.
HPIAF72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1860 as residues: Gln-1 to Arg-17, Ala-25 to Pro-32.
HPIAU01R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1861 as residues: Pro-9 to Gly-18.
HPIAU73R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1862 as residues: Arg-9 to Gln-35, Arg-51 to Gly-56.
HPIAW19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1863 as residues: Ala-16 to Arg-26, Thr-67 to Asn-76.
HPIAZ19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1864 as residues: Glu-1 to His-6, Gly-19 to Trp-31.
HPIBA31R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1865 as residues: Glu-1 to His-6, Gly-19 to Trp-31.
HPIBS06R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1866 as residues: Pro-25 to Lys-31.
HPICB65R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1867 as residues: Ser-2 to Gln-10, Val-26 to Lys-34, Asp-52 to Glu-58, Arg-93 to Trp-102.
HPJBF22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1868 as residues: Glu-1 to His-6, Gly-19 to Trp-31.
HPJBZ81R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1869 as residues: Ser-18 to Gly-23.
HSDJK57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1873 as residues: Thr-53 to Arg-64.
HSIFY54R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1874 as residues: Phe-35 to Asp-58, Phe-92 to Phe-105.
HUFAT72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1878 as residues: Pro-16 to Phe-25.
HULAI70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1879 as residues: Pro-13 to Gly-22, Arg-45 to Cys-50.
HTGFW12R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 1880 as residues: Pro-6 to Gly-16, Arg-24 to Pro-32.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in the deposited cDNA clone under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions, as defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention, and immunogenic and/or antigenic epitope fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light

- chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).
- 10 Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively,
- 15 deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See,
- 20 D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine

25 peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope

derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

- 5 Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.
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- Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the
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polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C- terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell

or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

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Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples

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of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most

preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether
5 directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition,
10 polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed
15 in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast
20 which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂.
25 Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast*

5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and

which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

- 10 In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if
- 15 as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid,
- 20 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L
- 25 (levorotary).

- Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see, e.g., Carter et al., *Nucl. Acids Res.* 13:4331 (1986); and Zoller et al., *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see, e.g., Wells et al., *Gene* 34:315
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(1985)), restriction selection mutagenesis (*see, e.g., Wells et al., Philos. Trans. R. Soc. London SerA 317:415 (1986)*).

The invention additionally, encompasses polypeptides of the present invention which are differentially modified during or after translation. *e.g.,* by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.,* N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (*see U.S. Patent No. 4,179,337*). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between

about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release
5 desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200; 500; 1000; 1500; 2000; 2500; 3000; 3500; 4000; 4500; 5000; 5500; 6000; 6500; 7000; 7500; 8000; 8500; 9000; 9500; 10,000;
10 10,500; 11,000; 11,500; 12,000; 12,500; 13,000; 13,500; 14,000; 14,500; 15,000; 15,500; 16,000; 16,500; 17,000; 17,500; 18,000; 18,500; 19,000; 19,500; 20,000; 25,000; 30,000; 35,000; 40,000; 50,000; 55,000; 60,000; 65,000; 70,000; 75,000; 80,000; 85,000; 90,000; 95,000; or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure.
15 Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconj. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

20 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting
25 pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues;
30 those having a free carboxyl group may include aspartic acid residues glutamic acid

residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

- 5 As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, 10 histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

- One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may 15 select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this 20 moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for 25 derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

- As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be 30 attached to the protein either directly or by an intervening linker. Linkerless systems

for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated
5 herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG,
10 polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of
15 different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-
20 2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference.
25 Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,
30 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of

substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The prostate cancer antigen polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X, and/or an amino acid sequence encoded by the cDNA in a related cDNA clone contained in a deposited library (including fragments, variants, splice variants, and fusion proteins, corresponding to any one of these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to

the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

- 5 Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers
- 10 of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides
- 15 of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or by the cDNA in the related cDNA clone contained in a deposited library). In one instance, the covalent associations are cross-linking between cysteine residues located within the
- 20 polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the
- 25 heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from
- 30 another protein that is capable of forming covalently associated multimers, such as for

example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention

containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

- 5 The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).
- 10 Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely
- 15 modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the
- 20 polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

- Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained
- 25 in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the
- 30 invention to a sequence encoding a linker polypeptide and then further to a synthetic
-

polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described
5 herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

10

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as
15 determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id
20 antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM,
25 IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and
30 fragments comprising either a VL or VH domain. Antigen-binding antibody

fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region.

- 5 CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from
- 10 human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

- The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for
- 15 different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920;
- 20 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

- Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size
- 25 in contiguous amino acid residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

- Antibodies of the present invention may also be described or specified in
- 30 terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog,

- or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.
- The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention
5 bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor
10 activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at
15 least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing
20 antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of
25 the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.
30 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.

- 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

- Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

- As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

- The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to a cellular ligand or other protein. etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin. etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire

or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999

(1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody

libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

- 5 Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the
- 10 human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous
- 15 deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention.
- 20 Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG,
- 25 IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European
- 30 Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825;

5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a

polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley &

Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence. for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art. e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions. e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived

from a murine mAb and a human immunoglobulin constant region. e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a

nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,

BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in

Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

- 5 In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo
- 10 recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the
- 15 ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate
- 20 transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

- In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g.,
- 25 cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which
- 30 possess the cellular machinery for proper processing of the primary transcript.
-

glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgpri- or apri- cells, respectively.

Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991);

- Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, 1993, *TIB TECH* 11(5):155-215; and hygro, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegl, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

- The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

- The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody

portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example,

- 5 Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053;
- 10 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

- 15 As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody
- 20 portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having
- 25 disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A
- 30 232,262). Alternatively, deleting the Fc part after the fusion protein has been

expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish

peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxel, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin, *A. pseudomonas* exotoxin, or diphtheria

- toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas
- 5 Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEG1 (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-
- 10 CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

- 15 Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp.
- 20 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*,
- 25 Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

- Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which
- 30 is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

5 ***Immunophenotyping***

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation. as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

- complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

- Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al. eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

- Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human

antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by

scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

5

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed
10 diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat,
15 inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is
20 not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the
25 present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes
30 without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic

acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

- 5 For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art
10 of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

- In a preferred aspect, the compound comprises nucleic acid sequences
15 encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment,
20 nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989). In
25 specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

- Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or
30 indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then

transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

- In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).
- In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which

facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy.

- 10 Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

- Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection

to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

- In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

- The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

- Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

- In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical

composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

- 10 Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of
- 15 introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together
- 20 with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such
- 25 as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

- In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion
- 30 during surgery, topical application, e.g., in conjunction with a wound dressing after

surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular. e.g., by

- use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection. or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliet et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

- The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of

the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

- For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

- The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

20

Diagnosis and Imaging

- Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide of interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹²⁵I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹²In), and technetium (⁹⁹Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval

following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that

5 detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

10 It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially

15 accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer. S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

20 Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5

25 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a

5 substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the

10 kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this

15 embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive

20 with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include

25 a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled

30 anti-human antibody to bind reporter to the reagent in proportion to the amount of

- bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

- The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support. such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

- Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Uses of the Polynucleotides

- Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

- The prostate cancer antigen polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X, or the complement thereto. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 3 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see. e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression,

chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention provides a method of detecting increased or decreased expression levels of the prostate cancer polynucleotides in affected individuals as compared to unaffected individuals using polynucleotides of the present invention and techniques known in the art, including but not limited to the method described in Example 11. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a prostate related disorder, including prostate cancer, involving measuring the expression level of prostate cancer polynucleotides in prostate tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard prostate cancer polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a prostate related disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'-mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a prostate related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed prostate cancer polynucleotide expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of prostate cancer polynucleotides" is intended qualitatively or quantitatively measuring or estimating the level of the prostate cancer polypeptide or the level of the mRNA encoding the prostate cancer polypeptide in a first biological sample either directly (e.g., by determining or
5 estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the prostate cancer polypeptide level or mRNA level in a second biological sample). Preferably, the prostate cancer polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard prostate cancer
10 polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the prostate related disorder or being determined by averaging levels from a population of individuals not having a prostate related disorder. As will be appreciated in the art, once a standard prostate cancer polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains prostate cancer polypeptide or the corresponding mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the prostate cancer polypeptide, prostate tissue, and other
20 tissue sources found to express the prostate cancer polypeptide. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic
25 method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with prostate cancer polynucleotides attached may be used to identify polymorphisms between the prostate cancer polynucleotide
30 sequences, with polynucleotides isolated from a test subject. The knowledge of such

polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, though most preferably in prostate related proliferative, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses prostate cancer polynucleotides that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ($T_{sub.m}$) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can

be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of

5 pathologically cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic

10 myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute

15 Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood. Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more

20 actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and

25 carcinoma. (Gelmann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of

30 HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-

myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a prostate cancer antigen polynucleotide can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA. while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman

and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to prostate or prostate cancer polynucleotides prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, prostate and prostate cancer tissues and/or cancerous and/or wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby

an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{152}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru .

luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels
5 or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody
10 by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$, (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium
15 (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F , ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It
20 will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of
25 cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi , or other radioisotopes such as, for example, ^{103}Pd , ^{133}Xe , ^{131}I , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{35}S , ^{90}Y , ^{153}Sm , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{113}Sn , $^{90}\text{Yttrium}$, ^{117}Tin , $^{186}\text{Rhenium}$, $^{166}\text{Holmium}$, and $^{188}\text{Rhenium}$; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the

use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

- 5 Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a prostate cancer polypeptide of the present invention in cells or body fluid of an individual, or more preferably, assaying the expression level of a prostate cancer polypeptide of the present invention in prostate cells or semen of an individual; and (b) comparing the assayed polypeptide
- 10 expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for
- 15 detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

- Moreover, prostate cancer antigen polypeptides of the present invention can
- 20 be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, preferably proliferative disorders of the prostate, and/or cancerous disease and
- 25 conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the
- 30 activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a

membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

- 5 Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, 10 such as by binding to a polypeptide bound to a membrane (receptor).

- At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from 15 a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

- 20 Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present 25 invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

- Thus, for example, cells from a patient may be engineered with a 30 polynucleotide (DNA or RNA) comprising a promoter operably linked to a

polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Beldegrun, A., et al., *J. Natl. Cancer Inst.* 85: 207-216 (1993); Ferrantini, M. et al., *Cancer Research* 53: 1107-1112 (1993); Ferrantini, M. et al., *J. Immunology* 153: 4604-4615 (1994); Kaido, T., et al., *Int. J. Cancer* 60: 221-229 (1995); Ogura, H., et al., *Cancer Research* 50: 5102-5106 (1990); Santodonato, L., et al., *Human Gene Therapy* 7:1-10 (1996); Santodonato, L., et al., *Gene Therapy* 4:1246-1255 (1997); and Zhang, J.-F. et al., *Cancer Gene Therapy* 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO.

pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

- 5 Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human
10 globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.
- 15 Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.
- 20 The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid,
25 mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for
30 the reasons discussed below. They may be conveniently delivered by injection into the

tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge

- complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

- Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxypropyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).
- Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

- Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta*

(1975) 394:483; Wilson et al., Cell (1979) 17:77); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta (1976) 443:629; Ostro et al., Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ratio will be from about 5:1 to about 1:5. More preferably, the ratio will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-

19-17-H2, RCRC, RCRCIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express a polypeptide of the present invention.

In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature

365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses.

cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra et al., *Nature* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, including direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

Preferably, the polynucleotide encoding a polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppository solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The

frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

- 5 Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

10 **Biological Activities**

- Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be
15 involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

Immune Activity

- 20 A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and
25 macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune
30 system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. Polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of

5 hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV

10 infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides or polypeptides, or agonists or antagonists of the

15 present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g.

20 thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of

30 polynucleotides or polypeptides, or agonists or antagonists of the present invention

that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, 5 rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic 10 Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides 15 or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host 20 disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, 25 particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention 30 may inhibit the proliferation and differentiation of cells involved in an inflammatory

- response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease. Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

- 10 Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or
- 15 antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated.

- 20 This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by

- 25 Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and
- 30 urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention

may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes" is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985)) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use

of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of

the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than $5 \times 10^{-6}M$, $10^{-6}M$, $5 \times 10^{-7}M$, $10^{-7}M$, $5 \times 10^{-8}M$, $10^{-8}M$, $5 \times 10^{-9}M$, $10^{-9}M$, $5 \times 10^{-10}M$, $10^{-10}M$, $5 \times 10^{-11}M$, $10^{-11}M$, $5 \times 10^{-12}M$, $10^{-12}M$, $5 \times 10^{-13}M$, $10^{-13}M$, $5 \times 10^{-14}M$, $10^{-14}M$, $5 \times 10^{-15}M$, and $10^{-15}M$.

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L. et al.,

Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K. et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous

- polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

- Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

- Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of Fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilog of Fallot, ventricular heart septal defects.

- Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right

- ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiectomy syndrome, pulmonary heart disease, rheumatic heart disease,
- 5 ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

- Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia,
- 10 Torsades de Pointes, and ventricular tachycardia.

- Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve
- 20 insufficiency, and tricuspid valve stenosis.

- Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial
- 25 reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

- Cardiovascular diseases also include vascular diseases such as aneurysms,
- 30 angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease.

- Klippel-Trenaunay-Weber Syndrome. Sturge-Weber Syndrome, angioneurotic edema. aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy. embolisms. thrombosis. erythromelalgia.
- 5 hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, ataxia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous
- 10 insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

- Arterial occlusive diseases include arteriosclerosis, intermittent claudication,
- 15 carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

- Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral
- 20 arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache,
- 25 migraine, and vertebrobasilar insufficiency.

- Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis,
- 30 Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, 5 thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

10 Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or 15 topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

20 Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound 25 healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. 30 A number of serious diseases are dominated by abnormal neovascularization

including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The polynucleotides encoding a polypeptide of the present invention may be administered along with other polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage stimulating factor, and nitric oxide synthase.

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists

may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; atherosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization;

telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalm.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalm.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however,

capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal

5 neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

10 Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions,

15 prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in

20 corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

25 Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbal corneal injection to "protect" the

30 cornea from the advancing blood vessels. This method may also be utilized shortly

after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The

compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Weber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or antagonists may also be used in controlling menstruation or administered as either a

peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch
5 granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal
10 surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention. compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic
15 compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-
20 angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the
25 site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly

preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo

molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

- A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., *Cancer Res.* 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, D,L-3,4-dehydroproline, Thiaproline, alpha, alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., *J. Bio. Chem.* 267:17321-17326, 1992); Chymostatin (Tomkinson et al., *Biochem J.* 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., *Nature* 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, *J. Clin. Invest.* 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., *J. Biol. Chem.* 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., *Agents Actions* 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma,

- lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

- Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis)
- myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

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Wound Healing and Epithelial Cell Proliferation

- In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound

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healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentop graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of

epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the

production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associated with the under expression.

- 5 Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent
- 10 or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as
- 15 agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

- Polynucleotides or polypeptides, as well as agonists or antagonists of the
- 20 present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

- 25 In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate,
- 30 delay or prevent permanent manifestation of the disease. Also, polynucleotides or

polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

5 **Neurological Diseases**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to
10 stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

- 15 Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain
20 edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph
25 Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis. cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease, cerebral
30 amyloid angiopathy, cerebral aneurysm. cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases. cerebral embolism and

- thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency,
- 5 vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache, migraine, dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis
- 10 which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular
- 15 leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, Hallervorden-Spatz Syndrome, hydrocephalus such as
- 20 Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia
- 25 Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, cerebral malaria, meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis. Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis,
- 30 Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome,

- Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uve-meningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and
- 5 postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie) cerebral toxoplasmosis, central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and
- 10 supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sclerolitis which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic
- 15 encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord
- 20 compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan
- 25 Syndrome, Maple Syrup Urine Disease, mucopolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes
- 30 hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele.
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- meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta, hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such
- 5 as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia,
- 10 broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium,
- 15 fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as
- 20 diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision
- 25 defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron
- 30 disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease

- and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome. Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica. Myotonia Confenita. Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome. Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2. Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome. ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis. ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome. Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis. Demyelinating Diseases such as Neuromyelitis Optica and Swayback, Diabetic neuropathies such as diabetic foot, nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Infectious Disease

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

- Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific

embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, *Aspergillosis*, *Bacillaceae* (e.g., *Anthrax*, *Clostridium*), *Bacteroidaceae*, *Blastomycosis*, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*, *Brucellosis*, *Candidiasis*, *Campylobacter*, *Coccidioidomycosis*, *Cryptococcosis*, *Dermatocycoses*, *E. coli* (e.g., *Enterotoxigenic E. coli* and *Enterohemorrhagic E. coli*), *Enterobacteriaceae* (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, *Legionellosis*, *Leptospirosis*, *Listeria*, *Mycoplasma*tales, *Mycobacterium leprae*, *Vibrio cholerae*, *Neisseriaceae* (e.g., *Acinetobacter*, *Gonorrhea*, *Menigococcal*), *Meisseria meningitidis*, *Pasteurellacea* Infections (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza* type B), *Pasteurella*), *Pseudomonas*, *Rickettsiaceae*, *Chlamydiaceae*, *Syphilis*, *Shigella* spp., *Staphylococcal*, *Meningiococcal*, *Pneumococcal* and *Streptococcal* (e.g., *Streptococcus pneumoniae* and Group B *Streptococcus*). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning,

- Typhoid. pneumonia, Gonorrhea. meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis. Diphtheria, Leprosy, Paratuberculosis. Tuberculosis. Lupus, Botulism, gangrene. tetanus, impetigo, Rheumatic Fever, Scarlet Fever. sexually transmitted diseases, skin diseases (e.g., cellulitis. dermatocycoses), toxemia. urinary tract infections. wound infections. Polynucleotides or polypeptides. agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, Polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphtheria, botulism. and/or meningitis type B.
- Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis. Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease. lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications. and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.
- Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient. supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases

(e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

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Chemotaxis

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit

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(antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan. et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention. after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process. eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458. and Patten. P. A., et al., *Curr. Opinion Biotechnol.* 8:724-

33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and ^3H

thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of ^3H thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of ^3H thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological

activity, and (b) determining if a biological activity of the polypeptide has been altered.

Targeted Delivery

5 In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

 As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or
10 prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic
15 protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

 In another embodiment, the invention provides a method for the specific
20 destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

 By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of
25 toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced
30 endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha

toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxycetamide derivatives of doxorubicin.

10 Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a

complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein.

Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the cDNA contained in the related cDNA clone identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J.,

Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

- 10 For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of
- 15 oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and
- 20 then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

- For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription
- 25 thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

- In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or
- 30 a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the

- invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors
- 5 can be plasmid, viral, or others known in the art. used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invention or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter
- 10 region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.
- 15 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the
- 20 RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a
- 25 stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

- Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work
- 30 most efficiently at inhibiting translation. However, sequences complementary to the
-

3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre et al., 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil.

- 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxycarboxymethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutosine, pseudouracil, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

- The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

- In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

- In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

- Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990)). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy

endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

Other Activities

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of

the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

5 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

10 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone
15 grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

20 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

25 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

5 A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of
10 energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or
15 Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals,
20 cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit,
25 goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in

the related cDNA clone contained in the deposit, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

- 5 Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of the cDNA in the related cDNA clone
10 contained in the deposit.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

- 15 Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule
20 comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete
25 nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence
30 selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the

complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit: which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X; or the cDNA in the related cDNA clone identified in Table 1 which encodes a protein. wherein the method comprises a step of detecting in a biological sample

obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for diagnosing a pathological condition which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 500, 1000, 2000, 3000 or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the cDNA clone referenced in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the

polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

- Also preferred is an isolated polypeptide comprising an amino acid sequence
- 5 at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

- Further preferred is an isolated polypeptide comprising an amino acid
- 10 sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

- Further preferred is an isolated polypeptide comprising an amino acid
- 15 sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

- Further preferred is an isolated polypeptide comprising an amino acid
- 20 sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

- Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of said polypeptide encoded by the cDNA clone referenced in Table 1; a polypeptide encoded by SEQ ID NO:X;
- 25 and/or the polypeptide sequence of SEQ ID NO:Y.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

- 5 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

10 Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

15 Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1; which method comprises a step of comparing an amino acid
20 sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

25 Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a
30 polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X;

and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in Table 1 encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a human protein comprising an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such

an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

- 5 Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprised administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of
- 10 said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

*Examples**Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample*

- 5 Each deposited cDNA clone is contained in a plasmid vector. Table 5 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The following correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 5 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
15	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
20	pCR [®] 2.1	pCR [®] 2.1

- Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Altting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Altting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS-. The S and K refers to the orientation of the polylinker to the T7 and T3

primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl ori of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lacmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR^{2.1}, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 5, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited by reference to Table 2 and 5 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone referenced in Table 1.

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLHF HLHG HLHH HLHQ			
HPMA HPMB HPNC HPMD HPME HPMF HPNG HPNH	Human Placenta	Uni-ZAP XR	LP03
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP03
HSIA HSIC HSID HSIE	Human Adult Small Intestine	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTEd HTEE HTEF HTEG HTEH HTEI HTEJ HTEK	Human Testes	Uni-ZAP XR	LP03
HTPA HTPB HTPC HTPD HTPe	Human Pancreas Tumor	Uni-ZAP XR	LP03
HTTA HTTB HTTC HTTD HTTE HTTF	Human Testes Tumor	Uni-ZAP XR	LP03
HAPa HAPB HAPC HAPM	Human Adult Pulmonary	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETE HETF HETG HETH HETI	Human Endometrial Tumor	Uni-ZAP XR	LP03
HHFB HHFC HHFD HHFE HHFF HHFG HHFH HHFI	Human Fetal Heart	Uni-ZAP XR	LP03
HHPB HHPC HHPD HHPE HHPF HHPG HHPI	Human Hippocampus	Uni-ZAP XR	LP03
HCEI HCE2 HCE3 HCE4 HCE5 HCEB HCEC HCED HCEE HCEF HCEG	Human Cerebellum	Uni-ZAP XR	LP03
HUVB HUVC HUVD HUVE	Human Umbilical Vein, Endo. rcmake	Uni-ZAP XR	LP03
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP03
HTAA HTAB HTAC HTAD HTAE	Human Activated T-Cells	Uni-ZAP XR	LP03
HFEA HFEB HFEC	Human Fetal Epithelium (Skin)	Uni-ZAP XR	LP03
HJPA HJPB HJPC HJPD	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Uni-ZAP XR	LP03
HESA	Human epithelioid sarcoma	Uni-Zap XR	LP03
HLTA HLTB HLTC HLTD HLTE HLTF	Human T-Cell Lymphoma	Uni-ZAP XR	LP03
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP03
HRDA HRDB HRDC HRDD HRDE HRDF	Human Rhabdomyosarcoma	Uni-ZAP XR	LP03
HCAA HCAB HCAC	Cem cells cyclohexamide treated	Uni-ZAP XR	LP03
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HSUA HSUB HSUC HSUM	Supt Cells, cyclohexamide treated	Uni-ZAP XR	LP03
HT4A HT4C HT4D	Activated T-Cells, 12 hrs.	Uni-ZAP XR	LP03
HE9A HE9B HE9C HE9D HE9E HE9F HE9G HE9H HE9M HE9N	Nine Week Old Early Stage Human	Uni-ZAP XR	LP03
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP03
HT5A	Activated T-Cells, 24 hrs.	Uni-ZAP XR	LP03
HFGA HFGM	Human Fetal Brain	Uni-ZAP XR	LP03
HNEA HNEB HNEC HNEd HNEE	Human Neutrophil	Uni-ZAP XR	LP03
HBGB HBGD	Human Primary Breast Cancer	Uni-ZAP XR	LP03
HBNA HBNB	Human Normal Breast	Uni-ZAP XR	LP03
HCA5	Cem Cells, cyclohexamide treated, subtra	Uni-ZAP XR	LP03
HHPS	Human Hippocampus, subtracted	pBS	LP03
HKCS HKCU	Human Colon Cancer, subtracted	pBS	LP03
HRGS	Raji cells, cyclohexamide treated, subtracted	pBS	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSUT	Supt cells. cyclohexamide treated. differentially expressed	pBS	LP03
HT4S	Activated T-Cells. 12 hrs. subtracted	Uni-ZAP XR	LP03
HCDA HCDB HCDC HCDD HCDE	Human Chondrosarcoma	Uni-ZAP XR	LP03
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP03
HTLA HTLB HTLC HTLD HTLE HTLF	Human adult testis, large inserts	Uni-ZAP XR	LP03
HLMA HLMC HLMD	Breast Lymph node cDNA library	Uni-ZAP XR	LP03
H6EA H6EB H6EC	HL-60, PMA 4H	Uni-ZAP XR	LP03
HTXA HTXB HTXC HTXD HTXE HTXF HTXG HTXH	Activated T-Cell (12hs)/Thiouridine labelledEco	Uni-ZAP XR	LP03
HNFA HNFb HNFc HNFd HNFf HNFf HNFg HNFh HNFj	Human Neutrophil. Activated	Uni-ZAP XR	LP03
HTOB HTOC	HUMAN TONSILS. FRACTION 2	Uni-ZAP XR	LP03
HMOB	Human OB MG63 control fraction 1	Uni-ZAP XR	LP03
HOPB	Human OB HOS control fraction 1	Uni-ZAP XR	LP03
HOBB	Human OB HOS treated (10 nM E2) fraction 1	Uni-ZAP XR	LP03
HSVA HSVB HSVC	Human Chronic Synovitis	Uni-ZAP XR	LP03
HROA	HUMAN STOMACH	Uni-ZAP XR	LP03
HBJA HBJB HBJC HBJD HBJE HBJF HBJG HBJH HBJI HBJJ HBJK	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP03
HCRA HCRB HCRC	human corpus colosum	Uni-ZAP XR	LP03
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP03
HDSA	Dermatofibrosarcoma Protuberance	Uni-ZAP XR	LP03
HMWA HMWB HMWC HMWD HMWE HMWF HMWG HMWH HMWI HMWJ	Bone Marrow Cell Line (RS4;11)	Uni-ZAP XR	LP03
HSOA	stomach cancer (human)	Uni-ZAP XR	LP03
HERA	SKIN	Uni-ZAP XR	LP03
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP03
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP03
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP03
HBCA HBCB	H. Lymph node breast Cancr	Uni-ZAP XR	LP03
HPWT	Human Prostate BPH. re-excision	Uni-ZAP XR	LP03
HFVG HFVH HFVI	Fetal Liver. subtraction II	pBS	LP03
HNFI	Human Neutrophils. Activated, re-excision	pBS	LP03
HBMB HBMC HBMD	Human Bone Marrow. re-excision	pBS	LP03
HKML HKMM HKMN	H. Kidney Medulla, re-excision	pBS	LP03
HKIX HKIY	H. Kidney Cortex, subtracted	pBS	LP03
HADT	H. Amygdala Depression, subtracted	pBS	LP03
H6AS	HL-60, untreated, subtracted	Uni-ZAP XR	LP03
H6ES	HL-60, PMA 4H, subtracted	Uni-ZAP XR	LP03
H6BS	HL-60, RA 4h, Subtracted	Uni-ZAP XR	LP03
H6CS	HL-60, PMA 1d, subtracted	Uni-ZAP XR	LP03
HTXJ HTXK	Activated T-cell(12h)/Thiouridine-re-	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	excision		
HMSA HMSB HMSC HMSD HMSE HMSF HMSG HMSH HMSI HMSJ HMSK	Monocyte activated	Uni-ZAP XR	LP03
HAGA HAGB HAGC HAGD HAGE HAGF	Human Amygdala	Uni-ZAP XR	LP03
HSRA HSRB HSRE	STROMAL - OSTEOCLASTOMA	Uni-ZAP XR	LP03
HSRD HSRF HSRG HSRH	Human Osteoclastoma Stromal Cells - unamplified	Uni-ZAP XR	LP03
HSQA HSQB HSQC HSQD HSQE HSQF HSQG	Stromal cell TF274	Uni-ZAP XR	LP03
HSKA HSKB HSKC HSKD HSKE HSKF HSKZ	Smooth muscle, serum treated	Uni-ZAP XR	LP03
HSLA HSLB HSLC HSLD HSLF HSLG HSLH	Smooth muscle, control	Uni-ZAP XR	LP03
HSDA HSDD HSDE HSDF HSDG HSDH	Spinal cord	Uni-ZAP XR	LP03
HPWS	Prostate-BPH subtracted II	pBS	LP03
HSKW HSKX HSKY	Smooth Muscle- HASTE normalized	pBS	LP03
HPFB HPFC HPFD	H. Frontal cortex, epileptic, re-excision	Uni-ZAP XR	LP03
HSDI HSDJ HSDK	Spinal Cord, re-excision	Uni-ZAP XR	LP03
HSKN HSKO	Smooth Muscle Serum Treated, Norm	pBS	LP03
HSKG HSKH HSKI	Smooth muscle, serum induced, re-exc	pBS	LP03
HFCA HFCB HFCC HFCD HFCE HFCF	Human Fetal Brain	Uni-ZAP XR	LP04
HPTA HPTB HPTD	Human Pituitary	Uni-ZAP XR	LP04
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP04
HE6B HE6C HE6D HE6E HE6F HE6G HE6S	Human Whole Six Week Old Embryo	Uni-ZAP XR	LP04
HSSA HSSB HSSC HSSD HSS E HSSF HSSG HSSH HSSI HSSJ HSSK	Human Synovial Sarcoma	Uni-ZAP XR	LP04
HE7T	7 Week Old Early Stage Human, subtracted	Uni-ZAP XR	LP04
HEPA HEPB HEPC	Human Epididymus	Uni-ZAP XR	LP04
HSNA HSNB HSN C HSNM HSN N	Human Synovium	Uni-ZAP XR	LP04
HPFB HPFC HPFD HPFE	Human Prostate Cancer, Stage C fraction	Uni-ZAP XR	LP04
HE2A HE2D HE2E HE2H HE2I HE2M HE2N HE2O	12 Week Old Early Stage Human	Uni-ZAP XR	LP04
HE2B HE2C HE2F HE2G HE2P HE2Q	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP04
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP04
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP04
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP04
HWTA HWTB HWTC	Wilms' tumor	Uni-ZAP XR	LP04
HBSD	Bone Cancr. re-excision	Uni-ZAP XR	LP04
HSGB	Salivary gland, re-excision	Uni-ZAP XR	LP04
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP04
HSXA HSXB HSXC HSXD	Human Substantia Nigra	Uni-ZAP XR	LP04
HSJA HSHB HS4C	Smooth muscle, IL1b induced	Uni-ZAP XR	LP04
HOUA HOUB HOUC HOUD HOUE	Adipocytes	Uni-ZAP XR	LP04

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP04
HELA HELB HELC HELD HELE HELF HELG HELH	Endothelial cells-control	Uni-ZAP XR	LP04
HEMA HEMB HEMC HEMD HEME HEMF HEMG HEMH	Endothelial-induced	Uni-ZAP XR	LP04
HBIA HBIB HBIC	Human Brain, Striatum	Uni-ZAP XR	LP04
HHSA HHSB HHSC HHSD HHSE	Human Hypothalamus, Schizophrenia	Uni-ZAP XR	LP04
HNGA HNGB HNGC HNGD HNGE HNGF HNGG HNGH HNGI HNGJ	neutrophils control	Uni-ZAP XR	LP04
HNHA HNHb HNHc HNHd HNHe HNHF HNHG HNHh HNHj HNHj	Neutrophils IL-1 and LPS induced	Uni-ZAP XR	LP04
HSDB HSDC	STRIATUM DEPRESSION	Uni-ZAP XR	LP04
HHPT	Hypothalamus	Uni-ZAP XR	LP04
HSAT HSAU HSAV HSAW HSAX HSAY HSAZ	Anergic T-cell	Uni-ZAP XR	LP04
HBMS HBMT HBMU HBMV HBMW HBMX	Bone marrow	Uni-ZAP XR	LP04
HOEA HOEB HOEC HOED HOEE HOEF HOEJ	Osteoblasts	Uni-ZAP XR	LP04
HAIA HAIB HAIC HAID HAIE HAIF	Epithelial-TNF α and INF induced	Uni-ZAP XR	LP04
HTGA HTGB HTGC HTGD	Apoptotic T-cell	Uni-ZAP XR	LP04
HMCA HMCB HMCC HMCD HMCE	Macrophage-oxLDL	Uni-ZAP XR	LP04
HMAA HMAB HMAc HMAD HMAE HMAF HMAG	Macrophage (GM-CSF treated)	Uni-ZAP XR	LP04
HPHA	Normal Prostate	Uni-ZAP XR	LP04
HPIA HPiB HPiC	LNCAP prostate cell line	Uni-ZAP XR	LP04
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP04
HOSE HOSF HOSG	Human Osteoclastoma, re-excision	Uni-ZAP XR	LP04
HTGE HTGF	Apoptotic T-cell, re-excision	Uni-ZAP XR	LP04
HMAJ HMAK	H Macrophage (GM-CSF treated), re-excision	Uni-ZAP XR	LP04
HACB HACC HACD	Human Adipose Tissue, re-excision	Uni-ZAP XR	LP04
HFPA	H. Frontal Cortex, Epileptic	Uni-ZAP XR	LP04
HFAA HFAB HFAC HFAD HFAE	Alzheimers, spongy change	Uni-ZAP XR	LP04
HFAM	Frontal Lobe, Dementia	Uni-ZAP XR	LP04
HMA HMIB HMIC	Human Manic Depression Tissue	Uni-ZAP XR	LP04
HTSA HTSE HTSF HTSG HTSH	Human Thymus	pBS	LP05
HPBA HPBB HPBC HPBD HPBE	Human Pituitary Gland	pBS	LP05
HSAA HSAB HSAC	HSA 172 Cells	pBS	LP05
HSBA HSBB HSBC HSBM	HSC172 cells	pBS	LP05
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBS	LP05
HJBA HJBB HJBC HJBD	Jurkat T-Cell, S phase	pBS	LP05
HAFA HAFB	Aorta endothelial cells + TNF- α	pBS	LP05
HAWA HAWB HAWC	Human White Adipose	pBS	LP05
HTNA HTNB	Human Thyroid	pBS	LP05
HONA	Normal Ovary, Premenopausal	pBS	LP05
HARA HARB	Human Adult Retina	pBS	LP05

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLJA HLJB	Human Lung	pCMVSPORT 1	LP06
HOFM HOFN HOFO	H. Ovarian Tumor. II. OV5232	pCMVSPORT 2.0	LP07
HOGA HOGB HOGC	OV 10-3-95	pCMVSPORT 2.0	LP07
HCGL	CD34+ cells, II	pCMVSPORT 2.0	LP07
HDLA	Hodgkin's Lymphoma I	pCMVSPORT 2.0	LP07
HDTA HDTB HDTC HDTD HDTE	Hodgkin's Lymphoma II	pCMVSPORT 2.0	LP07
HKAA HKAB HKAC HKAD HKAE HKAF HKAG HKAH	Keratinocyte	pCMVSPORT 2.0	LP07
HCIM	CAPFINDER, Crohn's Disease. lib 2	pCMVSPORT 2.0	LP07
HKAL	Keratinocyte. lib 2	pCMVSPORT 2.0	LP07
HKAT	Keratinocyte, lib 3	pCMVSPORT 2.0	LP07
HNDA	Nasal polyps	pCMVSPORT 2.0	LP07
HDRA	H. Primary Dendritic Cells.lib 3	pCMVSPORT 2.0	LP07
HOHA HOHB HOHC	Human Osteoblasts II	pCMVSPORT 2.0	LP07
HLDA HLDB HLDC	Liver, Hepatoma	pCMVSPORT 3.0	LP08
HLDN HLDO HLDP	Human Liver. normal	pCMVSPORT 3.0	LP08
HMTA	pBMC stimulated w/ poly I/C	pCMVSPORT 3.0	LP08
HNTA	NTERA2, control	pCMVSPORT 3.0	LP08
HDPA HDPB HDPC HDPD HDPE HDPG HDPH HDPI HDPJ HDPK	Primary Dendritic Cells. lib 1	pCMVSPORT 3.0	LP08
HDPM HDPN HDPO HDPP	Primary Dendritic cells.frac 2	pCMVSPORT 3.0	LP08
HMUA HMUB HMUC	Myeloid Progenitor Cell Line	pCMVSPORT 3.0	LP08
HHEA HHEB HHEC HHEE	T Cell helper I	pCMVSPORT 3.0	LP08
HHEM HHEN HHEO HHEP	T cell helper II	pCMVSPORT 3.0	LP08
HEQA HEQB HEQC	Human endometrial stromal cells	pCMVSPORT 3.0	LP08
HJMA HJMB	Human endometrial stromal cells-treated with progesterone	pCMVSPORT 3.0	LP08
HSWA HSWB HSWC	Human endometrial stromal cells-treated with estradiol	pCMVSPORT 3.0	LP08
HSYA HSYB HSYC	Human Thymus Stromal Cells	pCMVSPORT 3.0	LP08
HLWA HLWB HLWC	Human Placenta	pCMVSPORT 3.0	LP08
HRAA HRAB HRAC	Rejected Kidney. lib 4	pCMVSPORT 3.0	LP08
HMTM	PCR, pBMC I/C treated	PCR II	LP09
HMJA	H. Meningioma, M6	pSport 1	LP10
HMKA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport 1	LP10
HUSG HUSI	Human umbilical vein endothelial cells, IL-4 induced	pSport 1	LP10
HUSX HUSY	Human Umbilical Vein Endothelial Cells. uninduced	pSport 1	LP10
HOFA	Ovarian Tumor I, OV5232	pSport 1	LP10
HCFA HCFB HCFD HCFC	T-Cell PHA 16 hrs	pSport 1	LP10
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport 1	LP10
HADA HADC HADD HADE HADF HADG	Human Adipose	pSport 1	LP10
HOVA HOVB HOVC	Human Ovary	pSport 1	LP10
HTWB HTWC HTWD HTWE HTWF	Resting T-Cell Library.II	pSport 1	LP10

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMMA	Spleen metastatic melanoma	pSport 1	LP10
HLVA HLYB HLYC HLYD HLYE	Spleen, Chronic lymphocytic leukemia	pSport 1	LP10
HCGA	CD34+ cell. 1	pSport 1	LP10
HEOM HEON	Human Eosinophils	pSport 1	LP10
HTDA	Human Tonsil, Lib 3	pSport 1	LP10
HSPA	Salivary Gland, Lib 2	pSport 1	LP10
HCHA HCHB HCHC	Breast Cancer cell line, MDA 36	pSport 1	LP10
HCHM HCHN	Breast Cancer Cell line, angiogenic	pSport 1	LP10
HCIA	Crohn's Disease	pSport 1	LP10
HDAA HDAB HDAC	HEL cell line	pSport 1	LP10
HABA	Human Astrocyte	pSport 1	LP10
HUFA HUFB HUF C	Ulcerative Colitis	pSport 1	LP10
HNTM	NTERA2 + retinoic acid, 14 days	pSport 1	LP10
HDQA	Primary Dendritic cells, CapFinder2, frac 1	pSport 1	LP10
HDQM	Primary Dendritic Cells, CapFinder, frac 2	pSport 1	LP10
HLDX	Human Liver, normal, CapFinder	pSport 1	LP10
HULA HULB HULC	Human Dermal Endothelial Cells, untreated	pSport 1	LP10
HUMA	Human Dermal Endothelial cells, treated	pSport 1	LP10
HCAJ	Human Stromal Endometrial fibroblasts, untreated	pSport 1	LP10
HCMJ	Human Stromal endometrial fibroblasts, treated w/ estradiol	pSport 1	LP10
HEDA	Human Stromal endometrial fibroblasts, treated with progesterone	pSport 1	LP10
HFNA	Human ovary tumor cell OV350721	pSport 1	LP10
HKGA HKGB HKGC HKGD	Merkel Cells	pSport 1	LP10
HISA HISB HISC	Pancreas Islet Cell Tumor	pSport 1	LP10
HLSA	Skin, burned	pSport 1	LP10
HBZA	Prostate, BPH, Lib 2	pSport 1	LP10
HBZS	Prostate BPH, Lib 2, subtracted	pSport 1	LP10
HFIA HFIB HFIC	Synovial Fibroblasts (control)	pSport 1	LP10
HFIH HFII HFJ	Synovial hypoxia	pSport 1	LP10
HFIT HFIV HFV	Synovial IL-1/TNF stimulated	pSport 1	LP10
HGCA	Mesangial cell, frac 1	pSport 1	LP10
HMVA HMYB HMVC	Bone Marrow Stromal Cell, untreated	pSport 1	LP10
HFIX HFY HFIZ	Synovial Fibroblasts (III/TNF), sub	pSport 1	LP10
HFOX HFOY HFOZ	Synovial hypoxia-RSF subtracted	pSport 1	LP10
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP11
HLIA HLIB HLIC	Human Liver	pCMV Sport 1	LP012
HHBA HHBB HHBC HHBD HHBE	Human Heart	pCMV Sport 1	LP012
HBBA HBBB	Human Brain	pCMV Sport 1	LP012
HLJA HLJB HLJC HLJD HLJE	Human Lung	pCMV Sport 1	LP012
HOGA HOGB HOGC	Ovarian Tumor	pCMV Sport 2.0	LP012

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTJM	Human Tonsils, Lib 2	pCMVSPORT 2.0	LP012
HAMF HAMG	KMH2	pCMVSPORT 3.0	LP012
HAAJ HAJB HAJC	L428	pCMVSPORT 3.0	LP012
HWBA HWBB HWBC HWBD HWBE	Dendritic cells, pooled	pCMVSPORT 3.0	LP012
HWAA HWAB HWAC HWAD HWAE	Human Bone Marrow, treated	pCMVSPORT 3.0	LP012
HYAA HYAB HYAC	B Cell lymphoma	pCMVSPORT 3.0	LP012
HWHG HWHH HWHI	Healing groin wound, 6.5 hours post incision	pCMVSPORT 3.0	LP012
HWHF HWHQ HWHR	Healing groin wound: 7.5 hours post incision	pCMVSPORT 3.0	LP012
HARM	Healing groin wound - zero hr post-incision (control)	pCMVSPORT 3.0	LP012
HBIM	Olfactory epithelium: nasaleavity	pCMVSPORT 3.0	LP012
HWDA	Healing Abdomen wound: 70&90 mm post incision	pCMVSPORT 3.0	LP012
HWEA	Healing Abdomen Wound:15 days post incision	pCMVSPORT 3.0	LP012
HWJA	Healing Abdomen Wound:21&29 days	pCMVSPORT 3.0	LP012
HNAL	Human Tongue, frac 2	pSport1	LP012
HMJA	H. Meningioma, M6	pSport1	LP012
HMKAA HMKB HMKC HMKD HMKE	H. Meningioma, M1	pSport1	LP012
HOFA	Ovarian Tumor 1, OV5232	pSport1	LP012
HCFA HCFB HCFD HCFE	T-Cell PHA, 16 hrs	pSport1	LP012
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport1	LP012
HMMMA HMMB HMMC	Spleen metastatic melanoma	pSport1	LP012
HTDA	Human Tonsil, Lib 3	pSport1	LP012
HDBA	Human Fetal Thymus	pSport1	LP012
HDUA	Pericardium	pSport1	LP012
HBZA	Prostate.BPH, Lib 2	pSport1	LP012
HWCA	Larynx tumor	pSport1	LP012
HWKA	Normal lung	pSport1	LP012
HSMB	Bone marrow stroma,treated	pSport1	LP012
HBHM	Normal trachea	pSport1	LP012
HLFC	Human Larynx	pSport1	LP012
HLRB	Siebben Polyposis	pSport1	LP012
HNIA	Mammary Gland	pSport1	LP012
HNJB	Palate carcinoma	pSport1	LP012
HNKA	Palate normal	pSport1	LP012
HMZA	Pharynx carcinoma	pSport1	LP012
HABG	Cheek Carcinoma	pSport1	LP012
HMZM	Pharynx Carcinoma	pSport1	LP012
HDRM	Larynx Carcinoma	pSport1	LP012
HVAA	Pancreas normal PCA4 No	pSport1	LP012
HICA	Tongue carcinoma	pSport1	LP012
HUKA HUKB HUKC HUKD HUKF	Human Uterine Cancer	Lambda ZAP II	LP013
HFFA	Human Fetal Brain, random primed	Lambda ZAP II	LP013
HTUA	Activated T-cell labeled with 4-thiolur	Lambda ZAP II	LP013
HBOA	Early Stage Human Brain, random primed	Lambda ZAP II	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMEB	Human microvascular Endothelial cells, fract. B	Lambda ZAP II	LP013
HUSH	Human Umbilical Vein Endothelial cells, fract. A, re-excision	Lambda ZAP II	LP013
HLQC HLQD	Hepatocellular tumor, re-excision	Lambda ZAP II	LP013
HTWJ HTWK HTWL	Resting T-cell, re-excision	Lambda ZAP II	LP013
HF6S	Human Whole 6 week Old Embryo (II), sub1	pBluescript	LP013
HHPS	Human Hippocampus, subtracted	pBluescript	LP013
HLIS	LNCAP, differential expression	pBluescript	LP013
HLHS HLHT	Early Stage Human Lung, Subtracted	pBluescript	LP013
HSUS	Supt cells, cyclohexamide treated, subtracted	pBluescript	LP013
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBluescript	LP013
HSDS	H. Striatum Depression, subtracted	pBluescript	LP013
HPTZ	Human Pituitary, Subtracted VII	pBluescript	LP013
HSDX	H. Striatum Depression, sub1 II	pBluescript	LP013
HSZ	H. Striatum Depression, sub1	pBluescript	LP013
HPBA HPBB HPBC H1PBD HPBE	Human Pincal Gland	pBluescript SK-	LP013
HRTA	Colorectal Tumor	pBluescript SK-	LP013
HSBA HSBH HSBG HSBM	HSC172 cells	pBluescript SK-	LP013
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBluescript SK-	LP013
HJBA HJBB HJBC HJBD	Jurkat T-cell, S1 phase	pBluescript SK-	LP013
HTNA HTNB	Human Thyroid	pBluescript SK-	LP013
HAHA HAHB	Human Adult Heart	Uni-ZAP XR	LP013
HIE6A	Whole 6 week Old Embryo	Uni-ZAP XR	LP013
HFCB HFCE HFCC HFCD HFCE	Human Fetal Brain	Uni-ZAP XR	LP013
HFKE HFCD HFKE HFKE HFKE	Human Fetal Kidney	Uni-ZAP XR	LP013
HGBA HGBD HGBE HGBF HGBG	Human Gall Bladder	Uni-ZAP XR	LP013
HPRB HPRC HPRD HPRD	Human Prostate	Uni-ZAP XR	LP013
HTEA HTEB HTEC HTED HTEE	Human Testes	Uni-ZAP XR	LP013
HITTA HTTB HTTC HTTD HTTE	Human Testes Tumor	Uni-ZAP XR	LP013
HYBA HYBB	Human Fetal Bone	Uni-ZAP XR	LP013
HFLA	Human Fetal Liver	Uni-ZAP XR	LP013
HHFB HHFC HHFD HHFE HHFF	Human Fetal Heart	Uni-ZAP XR	LP013
HUVB HUVF HUVD HUVF	Human Umbilical Vein, End. remake	Uni-ZAP XR	LP013
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP013
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP013
HTAA HTAB HTAC HTAD HTAE	Human Activated T-cells	Uni-ZAP XR	LP013
HFEA HFEB HFEC	Human Fetal Epithelium (skin)	Uni-ZAP XR	LP013
HJPA HJPB HJPC HJPD	Human Jurkat Membrane Bound Polysomes	Uni-ZAP XR	LP013
HESA	Human Epithelioid Sarcoma	Uni-ZAP XR	LP013
HALS	Human Adult Liver, Subtracted	Uni-ZAP XR	LP013
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP013
HCAA HCAB HCAC	Cem cells, cyclohexamide treated	Uni-ZAP XR	LP013
HARGA HRGB HRCG HIRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP013
HE9A HE9B HE9C HE9D HE9E	Nine Week Old Early Stage Human	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HISFA	Human Fibrosarcoma	Uni-ZAP XR	LP013
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP013
HTRA	Human Trachea Tumor	Uni-ZAP XR	LP013
HE2A HE2D HE2E HE2H HE2I	12 Week Old Early Stage Human	Uni-ZAP XR	LP013
HE2B HE2C HE2F HE2G HE2P	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP013
HNEA HNEB HNEC HNEE HNEE	Human Neutrophil	Uni-ZAP XR	LP013
HJGA	Human Primary Breast Cancer	Uni-ZAP XR	LP013
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP013
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP013
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP013
HTOA HTOD HTOE HTOF HTOG	human tonsils	Uni-ZAP XR	LP013
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP013
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP013
HOQB	Human OB HOS treated (1 nM E2) fraction I	Uni-ZAP XR	LP013
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP013
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP013
HROA HROC	HUMAN STOMACH	Uni-ZAP XR	LP013
HBJA HBJB HBJC HBJD HBJE	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP013
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP013
HCPA	Corpus Callosum	Uni-ZAP XR	LP013
HISOA	stomach cancer (human)	Uni-ZAP XR	LP013
HIERA	SKIN	Uni-ZAP XR	LP013
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP013
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP013
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP013
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP013
HAPN HAPO HAPQ HAPR	Human Adult Pulmonary.re-excision	Uni-ZAP XR	LP013
HLTG HLTH	Human T-cell lymphoma.re-excision	Uni-ZAP XR	LP013
HAHC HAHD HAHE	Human Adult Heart.re-excision	Uni-ZAP XR	LP013
HAGA HAGB HAGC HAGD HAGE	Human Amygdala	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP013
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP013
HPJA HPJB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP013
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP013
HETA	Bone Marrow Stromal, TNF&LPS ind	Uni-ZAP XR	LP013
HMCJ HMCB HMCCH HMCI HMCJ	Macrophage-oxLDL: re-excision	Uni-ZAP XR	LP013
HAGG HAGH HAGI	Human Amygdala.re-excision	Uni-ZAP XR	LP013
HACA	H. Adipose Tissue	Uni-ZAP XR	LP013
HKFB	K562 + PMA (36 hrs).re-excision	ZAP Express	LP013
HCWT HCWU HCWV	CD34 positive cells (cord blood).re-ex	ZAP Express	LP013
HBWA	Whole brain	ZAP Express	LP013
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP013
HAVM	Temporal cortex-Alzheimer	pT-Adv	LP014
HAVT	Hippocampus, Alzheimer Subtracted	pT-Adv	LP014

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HHAS	CHME Cell Line	Uni-ZAP XR	LP014
HIAJR	Larynx normal	pSport 1	LP014
HWLE HWLF HWLG HWLH	Colon Normal	pSport 1	LP014
HCRM HCRN HCRO	Colon Carcinoma	pSport 1	LP014
HWLI HWLJ HWLK	Colon Normal	pSport 1	LP014
HWLQ HWLR HWLS HWLT	Colon Tumor	pSport 1	LP014
HBFM	Gastrocnemius Muscle	pSport 1	LP014
HBOD HBOE	Quadriceps Muscle	pSport 1	LP014
HBKD HBKE	Soleus Muscle	pSport 1	LP014
HCCM	Pancreatic Langerhans	pSport 1	LP014
HWGA	Larynx carcinoma	pSport 1	LP014
HWGM HWGN	Larynx carcinoma	pSport 1	LP014
HWLA HWLB HWLC	Normal colon	pSport 1	LP014
HWLM HWLN	Colon Tumor	pSport 1	LP014
HVAM HVAN HVAO	Pancreas Tumor	pSport 1	LP014
HWGO	Larynx carcinoma	pSport 1	LP014
HAAQ HAAQ	Salivary Gland	pSport 1	LP014
HASM	Stomach: normal	pSport 1	LP014
HBCM	Uterus: normal	pSport 1	LP014
HCDM	Testis: normal	pSport 1	LP014
HJDM	Brain: normal	pSport 1	LP014
HEFM	Adrenal Gland: normal	pSport 1	LP014
HBAA	Rectum normal	pSport 1	LP014
HFDM	Rectum tumour	pSport 1	LP014
HGAM	Colon: normal	pSport 1	LP014
HHMM	Colon: tumour	pSport 1	LP014
HCLB HCLC	Human Lung Cancer	Lambda Zap II	LP015
HRLA	IL1 Cell line	ZAP Express	LP015
HHAM	Hypothalamus, Alzheimer's	pCMV Sport 3.0	LP015
HKBA	Ku 812F Basophils Line	pSport 1	LP015
HS2S	Saas2, Dexamethosone Treated	pSport 1	LP016
HASA	Lung Carcinoma A549 TNFalpha activated	pSport 1	LP016
HTFM	TF-1 Cell Line GM-CSF Treated	pSport 1	LP016
HYAS	Thyroid Tumour	pSport 1	LP016
HUTS	Larynx Normal	pSport 1	LP016
HIXOA	Larynx Tumor	pSport 1	LP016
HEAH	Ea.hy 926 cell line	pSport 1	LP016
HINA	Adenocarcinoma Human	pSport 1	LP016
HRMA	Lung Mesothelium	pSport 1	LP016
HLCL	Human Pre-Differentiated Adipocytes	Uni-Zap XR	LP017
HSZA	Saas2 Cells	pSport 1	LP020
HS2I	Saas2 Cells: Vitamin D3 Treated	pSport 1	LP020
HUCM	CHME Cell Line, untreated	pSport 1	LP020
HEPN	Aryepiglottis Normal	pSport 1	LP020
HPSN	Sinus Pinfornis Tumour	pSport 1	LP020
HNSA	Stomach Normal	pSport 1	LP020

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HNSM	Stomach Tumour	pSport 1	LP020
HNLA	Liver Normal Met5No	pSport 1	LP020
HUTA	Liver Tumour Met 5 Tu	pSport 1	LP020
HOCN	Colon Normal	pSport 1	LP020
HOCT	Colon Tumour	pSport 1	LP020
HTNT	Tongue Tumour	pSport 1	LP020
HLXN	Larynx Normal	pSport 1	LP020
HLXT	Larynx Tumour	pSport 1	LP020
HTYN	Thymus	pSport 1	LP020
HPLN	Placenta	pSport 1	LP020
HTNG	Tongue Normal	pSport 1	LP020
HZAA	Thyroid Normal (SDCA2 No)	pSport 1	LP020
HWES	Thyroid Thyroiditis	pSport 1	LP020
HFHD	Ficollised Human Stromal Cells, 5Fu treated	pTriplEx2	LP021
HFHM,HFHN	Ficollised Human Stromal Cells, Untreated	pTriplEx2	LP021
HPCT	Hep G2 Cells, lambda library	lambda Zap-CMV XR	LP021
HBCA,HBCB,HBC	H. Lymph node breast Cancer	Uni-ZAP XR	LP021
HCOK	Chondrocytes	pSPORT1	LP022
HDCA, HDCB, HDCC	Dendritic Cells From CD34 Cells	pSPORT1	LP022
HDMA, HDMB	CD40 activated monocyte dendritic cells	pSPORT1	LP022
HDDM, HDDN, HDDO	LPS activated derived dendritic cells	pSPORT1	LP022
HPCR	Hep G2 Cells, PCR library	lambda Zap-CMV XR	LP022
HAAA, HAAB, HAAAC	Lung, Cancer (4005313A3): Invasive Poorly Differentiated Lung Adenocarcinoma	pSPORT1	LP022
HIPA, HIPB, HIPC	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	pSPORT1	LP022
HOOH, HOOI	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	pSPORT1	LP022
HIDA	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HUJA,HUJB,HUJC,HUJD,HUJE	B-Cells	pCMV Sport 3.0	LP022
HNOA,HNOB,HNOC,HNOD	Ovary, Normal: (9805C040R)	pSPORT1	LP022
HNLM	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HSCL	Stromal Cells	pSPORT1	LP022
HAAX	Lung, Cancer: (4005313 A3) Invasive Poorly-differentiated Metastatic lung adenocarcinoma	pSPORT1	LP022
HUUA,HUUB,HUUC,HUUD	B-cells (unstimulated)	pTriplEx2	LP022
HWWA,HWWB,HWWC,HWWD,HWE,HWWF,HWWG	B-cells (stimulated)	pSPORT1	LP022
HCCC	Colon, Cancer: (9808C064R)	pCMV Sport 3.0	LP023
HPDO HPDP HPDQ HPDR HPD	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	pSport 1	LP023
HPCO HPCP HPCQ HPCT	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	pSport 1	LP023
HOCM HOCO HOCP HOCQ	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	pSport 1	LP023

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
IICBM HCBN HCBO	Breast, Cancer: (4004943 A5)	pSport 1	LP023
HNBT HNBH HNBV	Breast, Normal: (4005522B2)	pSport 1	LP023
HBCP HBCQ	Breast, Cancer: (4005522 A2)	pSport 1	LP023
HBCJ	Breast, Cancer: (9806C012R)	pSport 1	LP023
HSAM HSAN	Stromal cells 3.88	pSport 1	LP023
HVCA HVCB HVCC HVCD	Ovary, Cancer: (4004332 A2)	pSport 1	LP023
HSCK HSEN HSEO	Stromal cells (HBM3.18)	pSport 1	LP023
HSCP HSCQ	stromal cell clone 2.5	pSport 1	LP023
HUXA	Breast Cancer: (4005385 A2)	pSport 1	LP023
HCOM HCON HCOO HCOP HCOQ	Ovary, Cancer (4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	pSport 1	LP023
HBNM	Breast, Cancer: (9802C020E)	pSport 1	LP023
HVVA HVVB HVVC HVVD HVVE	Human Bone Marrow, treated	pSport 1	LP023

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 5. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence of SEQ ID NO:X.

- 5 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P - γ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid
- 10 mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using
- 15 Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- Alternatively, two primers of 17-20 nucleotides derived from both ends of the
- 20 nucleotide sequence of SEQ ID NO:X are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 μg of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl_2 , 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of
- 25 Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA
- 30 product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not

limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

5 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full
10 length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the
15 RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis
20 using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

25 *Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide*

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the sequence corresponding to SEQ ID NO:X, according to the method
30 described in Example 1. (See also. Sambrook.)

Example 3: Tissue specific expression analysis

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue specific cDNA libraries. Libraries generated from a particular tissue are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs which show tissue specific expression are selected.

The original clone from which the specific EST sequence was generated, is obtained from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured then transferred in 96 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of tissue specific clones. Housekeeping genes, maize genes, and known tissue specific genes are included on the filters. These targets can be used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

Radioactively labeled hybridization probes are generated by first strand cDNA synthesis per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed. The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified and the full length sequence of these clones is generated.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute

cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR
5 fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using
10 PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial
15 expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is
20 ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is
25 isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto
30 pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction

sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μ m membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Comassie blue stained 16% SDS-PAGE gel when 5 μ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the

polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five μ g of a plasmid containing the polynucleotide is co-transfected with 1.0 μ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc.*

Natl. Acad. Sci. USA 84:7413-7417 (1987). One μg of BaculoGold™ virus DNA and 5 μg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 μCi of ^{35}S -methionine and 5 μCi ^{35}S -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA. a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 or pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones

are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Trautnecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without

a stop codon. otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the polypeptide of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous
 5 signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

GGGATCCGGAGCCCAATCTTCTGACAAAACCTCACACATGCCACCGTGCCAG
 CACCTGAATTCGAGGGTGCACCGTCAGTCTTCTCTCCCCCAAAACCAAGGA
 10 CACCCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGTGGTGGACGTAAGC
 CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT
 AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
 AGCGTCTCTACCGTCTGCAACAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
 AAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGAAAACCATCTCCAAAGCC
 15 AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAG
 CTGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGC
 GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACAACTACAAGAC
 CACGCCTCCCGTGTGGACTCCGACGGTCTCTTCTCTCTACAGCAAGCTCACC
 GTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGATGCAT
 20 GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
 GAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1881)

Example 10: Production of an Antibody from a Polypeptide

25 a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide of the present invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide
 30 of the present invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide of the present invention are prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*. Elsevier, N.Y., pp. 563-681 (1981)). In
5 general, an animal (preferably a mouse) is immunized with polypeptide of the present invention or, more preferably, with a secreted polypeptide of the present invention-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino
10 acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention: however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in
15 HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention.

Alternatively, additional antibodies capable of binding to polypeptide of the present
20 invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma
25 cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide of the present invention-specific antibody can be blocked by polypeptide of the present invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide of the present invention-specific antibody and are used to immunize an animal to induce formation of further polypeptide of the present invention-specific antibodies.

30 For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized

antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Against Polypeptide of the Present Invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations

(Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μ m filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 μ g/ml or 10 μ g/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 μ g/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 μ g/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 11: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is isolated. cDNA is then generated from these RNA

samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X; and/or the nucleotide sequence of the related cDNA in the cDNA clone contained in a deposited library. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30
5 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products
10 analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected
15 individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991).
20 Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination
25 with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions,
30 deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 13: Formulation

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with

a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known *per se*: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, *CRC Crit. Rev. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not

include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is

lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention.

5 Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

10 The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific
15 embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to,
20 vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or
25 concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

30 The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF

family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the

invention, include, but are not limited to, VIRAMUNET™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and
5 VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in
10 combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™,
15 GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICLOVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™,
20 DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific
25 embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic
30 cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or

KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, 5 GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be 10 administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4- 15 hydroxybutyric acid, amixetrine, bendazac, benzydamine, buclome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be 20 administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, 25 cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and 30 combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in

combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and
5 any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment,
10 Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with
15 the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO
20 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular
25 Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as
30 disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in

combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

5 In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

10 In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 14: Method of Treating Decreased Levels of the Polypeptide

15 The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a polypeptide of the present invention in an individual can be treated by administering the
20 agonist or antagonist of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the agonist or antagonist to increase the activity level of the polypeptide in such an individual.

25 For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist or antagonist for six consecutive days. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 13.

Example 15: Method of Treating Increased Levels of the Polypeptide

30

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to

such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 13.

Example 16: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy.

The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a

HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then
5 plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the
10 media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the
15 infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low,
20 then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

25 *Example 17: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention*

Another method of gene therapy according to the present invention involves operably
30 associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996;

International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

5 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting
10 sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

15 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then
20 purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

25 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

30 Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An

aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells
5 resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3×10^6 cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the
10 invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a
15 HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The
20 final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5×10^6 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving
25 cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a
30 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having

been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 18: Method of Treatment Using Gene Therapy - In Vivo

5

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the

polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal. including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the

present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

5 Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is
10 placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein
15 expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and
20 other animals using naked DNA.

Example 19: Transgenic Animals

The polypeptides of the invention can also be expressed in transgenic animals.
25 Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

30 Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson

et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos;
5 gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol. Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene
10 transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals." Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of
15 nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as
20 multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those
25 of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of
30 the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences

required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 20: Knock-Out Animals

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson

et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent
5 No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the
10 immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with
15 aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 22: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

20

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date,
25 numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

30 One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses.

Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the
5 detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Agonists or antagonists of the invention can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention
10 on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell
15 proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to
20 which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2
mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive
this treatment for 4 consecutive days, at which time they are sacrificed and various tissues
and serum collected for analyses. Comparison of H&E sections from normal spleens and
spleens treated with agonists or antagonists of the invention identify the results of the activity
30 of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic
sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which
may indicate the activation of the differentiation and proliferation of B-cell populations.

Immunohistochemical studies using a B cell marker. anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

- 5 Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice. Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared
10 between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

15 *Example 23: T Cell Proliferation Assay*

- A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^3H -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 μl /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control
20 mAb (B33.1) overnight at 4 degrees C (1 $\mu\text{g}/\text{ml}$ in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4 /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists or antagonists of the invention (total volume 200 μl). Relevant protein buffer and medium
25 alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 μl of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 μl of medium containing 0.5 μCi of ^3H -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of ^3H -thymidine used as a measure of proliferation.
30 Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of agonists or antagonists of the

invention.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

5 *Example 24: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells*

10 Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- α , causes a rapid change in
15 surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC γ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive
20 control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

25 Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10⁶/ml) are treated with increasing concentrations of agonists or antagonists of the
30 invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols

provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml

TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5 μ g/ml, and then incubated at room temperature for 5 minutes before FACSscan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows.

- Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use.
- Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e. g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

- Oxidative burst. Purified monocytes are plated in 96-w plate at 2×10^5 cell/well. Increasing concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRP) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

- The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 25: Biological Effects of Agonists or Antagonists of the Invention

5 Astrocyte and Neuronal Assays.

Agonists or antagonists of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an agonist or antagonist of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an agonist or antagonist of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

25 Fibroblast and endothelial cell assays.

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal